The occurrence of antibiotic resistant bacteria in Swedish dairy products

– A pilot study

Förekomsten av antibiotikaresistenta bakterier i svenska mejeriprodukter - En pilotstudie

Viktor Nilsson

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Swedish University of Agricultural Sciences, SLU
Department of Molecular Sciences
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The occurrence of antibiotic resistant bacteria in Swedish dairy products – A pilot study

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Viktor Nilsson

Supervisor:	Monika Johansson, SLU, Department of Molecular Sciences
Assistant supervisor:	Li Sun, SLU, Department of Molecular Sciences
Examiner:	Åse Lundh, SLU, Department of Molecular Sciences

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Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Science
Department of Molecular Sciences

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Abstract

Antibiotics have been used in human and animal treatment for many years and revolutionized the modern medicine. Overconsumption and misuse have led to a global problem with antibiotic resistance. Sweden is among the countries in the European union with the lowest usage of antibiotic for livestock. Development and spread of antibiotic resistant bacteria in humans can occur through consumption of foods. The objective of this pilot study aimed to investigate the occurrence of antibiotic resistant bacteria in unpasteurized milk and Swedish dairy products (pasteurized milk, fresh cheese, ripened hard cheese and fermented milk) available on Swedish market. Culturing and isolation of bacteria was performed along with taxonomic identification using MALDI-TOF MS. Nine antibiotics were used for the investigation of bacterial resistance. These antibiotics belong to some of the most important antibiotic classes used in human and veterinary medicine. Eleven antibiotic resistant bacteria species were identified. Antibiotic resistant bacteria were found in unpasteurized milk and in all the dairy products. The bacteria species were resistant towards eight antibiotics included in the investigation. Greatest diversity of antibiotic resistant bacteria species was identified in unpasteurized milk and fermented milk. Antibiotic resistant lactic acid bacteria were found in ripened hard cheese, fermented milk and unpasteurized milk. This study used a single batch of each investigated product. Further research that includes more batches from each product is required in order to elucidate how frequently occurring antibiotic resistant bacteria are in Swedish dairy products.

Keywords: Antibiotics, antibiotic resistant bacteria, susceptible, resistant, zone diameter unpasteurized milk, pasteurized milk, fresh cheese, ripened hard cheese, fermented milk

Sammanfattning

Antibiotika har använts i behandling av människor och djur under många år och har revolutionerat modern medicin. Överkonsumtion och missbruk har lett till ett globalt problem med antibiotikaresistens. Sverige är bland de länder i Europeiska unionen som har den lägsta användningen av antibiotika för boskapsdjur. Utveckling och spridning av antibiotikaresistenta bakterier hos människor kan inträffa genom konsumtion av livsmedel. Målet med denna pilotstudie syftade till att undersöka förekomsten av antibiotikaresistenta bakterier i opastöriserad mjölk och svenska mejeriprodukter (pastöriserad mjölk, färskost, lagrad hårdost och fermenterad mjölk) som finns tillgängliga på den svenska marknaden. Odling och isolering av bakterier utfördes tillsammans med taxonomisk identifiering med MALDI-TOF MS. Nio antibiotika, tillhörande några av de viktigaste antibiotika som används inom human- och veterinärmedicin, användes för undersökning av bakterieresistens. Antibiotikaresistenta bakterier påträffades i opastöriserad mjölk och alla mejeriprodukter, och elva antibiotikaresistenta bakterier identifierades. Bakterierna var resistenta mot åtta av de antibiotika som inkluderades i undersökningen. Den största mångfalden av antibiotikaresistenta bakteriearter identifierades i opastöriserad mjölk och fermenterad mjölk. Antibiotikaresistenta mjölksyrabakterier hittades i lagrad hårdost, fermenterad mjölk och opastöriserad mjölk. Denna studie använde endast en batch från varje produkt. Ytterligare forskning som omfattar fler batcher av varje produkt krävs för att klargöra hur frekvent förekomsten av antibiotikaresistenta bakterier är i svenska mejeriprodukter.

Nyckelord: Antibiotika, antibiotikaresistenta bakterier, mottaglig, resistent, zondiameter opastöriserad mjölk, pastöriserad mjölk, färskost, lagrad hårdost, fermenterad mjölk

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Abbreviations

AMP	Ampicillin
ARB	Antibiotic resistant bacteria
C	Chloramphenicol
CN	Gentamicin
DA	Clindamycin
E	Erythromycin
HGT	Horizontal gene transfer
K	Kanamycin
LAB	Lactic acid bacteria
MH	Mueller-Hinton
MRS	De Man, Rogosa and Sharpe
MSPR	Mannitol Salt Phenol Red
MTP	Maldi Target Plate
M/Z	Mass to charge
S	Streptomycin
TE	Tetracycline
VA	Vancomycin
VRBD	Violet Red Bile Dextrose
ZD	Zone diameter

1. Introduction

Since the discovery of the penicillin by Sir Alexander Fleming in 1928 the first antibiotic entered the history. Antibiotic discoveries continued the years to come and have revolutionized modern medicine with the contribution of controlling bacterial infections in human health, livestock and agriculture (Munita and Arias, 2016; Garzón et al., 2020). However, since the 1980s only one major new class of antibiotics has been discovered and since the year 2000 only five new classes of antibiotics have been introduced on the market (OECD, 2016).

Unfortunately, the intensive usage of antibiotics worldwide in medicine and agriculture has dramatically increased, leading to increased frequency of antibiotic resistance among pathogens (Andersson and Hughes, 2010). Increased frequencies of antibiotic resistance reduce the possibility of effectively treating infections and prevent deaths in the future (Andersson and Hughes, 2011).

Only in the United States (US) about 47 million antibiotic courses are prescribed for outpatients each year for infections which could be treated differently. This amount corresponds to approximately 30% of all antibiotics prescribed (CDC, 2020). A report from the worldwide Organization for Economic Co-operation and Development (OECD) states that the inappropriate use of antibiotics among the 36 member states, Sweden included, may account for up to 50% of all antimicrobials consumed in health care (OECD, 2016). The OECD also states that the agricultural sector accounts for over 75% of the annual antimicrobial usage in EU and US. Antibiotics play an important role in ensuring animal welfare through treatment of infectious diseases, thus maintaining global food production (WHO, 2009). In addition, antibiotics used for livestock are frequently belonging to the same classes as those used in human medicine. Thereby increasing the risk of developing resistant bacteria that are carried in food-producing animals and spread to people via foods. The extensive use of antibiotics in dairy farms in the treatment of diseases such as mastitis had led to transfer of antibiotic resistant bacteria (ARB) to unpasteurized milk (Özdikmenli Tepeli and Demirel Zorba, 2018).

The volume of cow milk produced worldwide was by 2019 approximately 522 million metric tons, from which 155 million metric tons were generated in the European Union. This means that the region is the top producer of cow milk in the world (Shahbandeh, 2020). In Sweden, approximately 2.7 million metric tons of

cow milk was produced during 2019 of which 64% was used in the production of dairy food products (Jordbruksverket[1], 2020; LRF, 2020).

Lactic acid bacteria (LAB) occur naturally in milk and are commonly used as starter- and probiotic cultures in a variety of dairy food products (Widyastuti et al., 2014, Georgieva et al., 2015). The LAB has a Qualified Presumption of Safety (QPS) status by the European Food Safety Authority (EFSA). The QPS status means that the LAB do not pose any safety concern to humans. However, acquired genes for AR have been detected in LAB species isolated from foods, dairy products included (EFSA 2007, Kim et al., 2018, Chajęcka-Wierzchowska et al., 2020).

Concerns about transfer of ARB from food-producing animals to human microbiota led in 2006 to an EU-wide ban on the use of antibiotics as growth promoters in animal feed (Castanon, 2007). In Sweden, the use of antibiotics for growth promotion was prohibited in 1986. (Jordbruksverket[2], 2020). Current regulations state that antibiotics for animals are only available on prescription and can only be sold by pharmacies. However, regardless the more controlled use of antibiotics in Sweden they are still used for treatment of infections among food-producing animals, including dairy cows (Växa, 2019). This raises the question if ARB exist in milk and dairy products available on the Swedish market.

1.1. Purpose and objective

This project was designed as an explorative pilot study, with the purpose to make a screening of ARB in milk and dairy products. Thus, this project aimed to investigate the current status of the possible content of ARB in Swedish dairy products available on the Swedish market. The study included products based on bovine milk e.g., 1) pasteurized milk, 2) unpasteurized milk, 3) fermented cheese, 4) fresh cheese, 5) ripened hard cheese. The hypothesis was that the dairy products are of high quality for consumption and free from ARB.

1.2. Delimitations

This pilot study was limited to include no more than one single batch of each product, which also lowered the screening amount of possible ARB. Also, the number of antibiotics included in the screening was limited. Consequently, the results cannot be generalized as deeper study would be needed.

2. Background

2.1. Antibiotics

Antibacterial drugs (antibiotics) are molecules that are either bacteriostatic (inhibit bacterial replication or growth) or bactericidal (cause death or lysis of bacterial cell) and thus can be used to treat bacterial infectious diseases (Walsh, 2003). The discovery of antibiotics has underpinned modern medicine. Before the discovery of penicillin by Alexander Fleming, minor infections and diseases such as pneumonia often caused death due to septicemia (Ferri et al., 2017). Their subsequent application in clinical medicine has increased life expectancy and saved lives of millions of people through reduced mortality from bacterial infections (Zaman et al. 2017; Blair et al., 2015).

The primary source of antibiotics derives naturally from microorganisms (Awad et al., 2012). Almost all antibiotic activities from natural antibiotics are products of secondary metabolic pathways and have no artificial additives (Walsh, 2003). An example of such antibiotics are streptomycin and tetracycline obtained from *Streptomyces* species, gentamicin obtained from *Micromonospora purpurea* and penicillins obtained from *Penicillium* species (MicroDok, 2018). However, semi-synthetic antibiotics are natural antibiotics that are chemically modified in order to increase the therapeutic efficacy of the drug and to minimize the side effects. Example of a semi-synthetic antibiotic is ampicillin. Antibiotics which are designed and produced in laboratories are categorized as synthetic antibiotics. With advances in medical science the synthetic antibiotics could target the crucial replication element of protein synthesis and block the process before its initiation. One example of such antibiotics is chloramphenicol (Awad et al., 2012).

Classifications of antibiotics that are most widely used today is based on the antibiotic spectrum, mechanism of action and chemical structure (Garzón et al., 2020).

2.1.1. Antibiotics spectra

The biological activity of antibiotics can be separated into broad-spectrum and narrow-spectrum antibiotics. Broad-spectrum antibiotics act in a wide range on

gram-positive and gram-negative bacteria (Awad et al., 2012). In narrow-spectrum antibiotics bacteriostatic and bactericidal ability is limited, thus they are only effective against certain types of organisms or infections

2.1.2. Mechanism of action

The activity of an antibiotic develops through a limited number of mechanisms of action where some of the major once are described beneath.

Inhibition of bacterial cell wall biosynthesis

Both gram-positive and -negative bacteria have a cell wall layer consisting of long sugar polymers called peptidoglycan. The peptidoglycan undergoes enzymatic crosslinking of the glycan and peptide strands by transglycosylase and transpeptidase action, respectively, thus maintaining the shape and mechanical strength of the cell wall (Kapoor et al., 2017; Walsh, 2003). Antibiotics that affect the bacterial cell wall block the biosynthesis of peptidoglycan by inhibiting enzymes or sequester substrates involved in the peptidoglycan assembly and cross-linking (Kohanski et al., 2010). Hence, the bacterial cells become distorted and osmotic imbalance will lead to cell lysis. Antibiotics involved in this mechanism includes glycopeptides, e.g. vancomycin, and β-lactams, e.g. ampicillin.

Inhibition of protein biosynthesis

One of the most common target mechanisms of the antibiotics is the protein biosynthesis (Garzón et al., 2020). In bacteria the 70S ribosome is composed of two subunit nucleoprotein particles. The small subunit 30S and the large subunit 50S synthesize proteins present in m-RNA during the translation process (Kapoor et al., 2017). Antibiotics inhibit protein biosynthesis by binding to receptors on either the 30S or 50S subunit. After initial binding to the ribosome, the reaction is leading to inhibition of the activation phase of the proteins, onset of protein synthesis and preventing the formation of translation elements (Garzón et al., 2020). These disruptions will slow down growth or be lethal to the bacteria. Antibiotics that act as protein biosynthesis inhibitors include tetracycline, kanamycin and streptomycin targeting the 30S subunit and clindamycin, chloramphenicol and erythromycin targeting the 50S subunit (Walsh, 2003).

Inhibition of DNA synthesis function

The enzymes called topoisomerases are essential for cell viability in both prokaryotic and eukaryotic cells through their involvement in the processes of replication, transcription and translation of nucleic acids (Garzón et al., 2020, Walsh, 2003). Antibiotics mainly pinpoint the enzymes topoisomerase II (DNA gyrase) in gram-negative bacteria and topoisomerase IV in gram-positive bacteria, thereby inhibiting synthesis of DNA in bacterial cells (Kohanski et al., 2010).

Antibiotics involved in this mechanism includes quinolones, rifampicin and sulphonamides (MicroDok, 2018).

Other targets of antibiotics

Antibiotics are also able to disrupt the bacterial cytoplasmic membrane (Garzón et al., 2020). Lipopeptide antibiotics can generate irreversible influx or outflux of ions through altering the structure of the bacterial membrane and changing its permeability. The integrity of the membrane will be disturbed which could lead to cell death. Further, antibiotics of sulphonamides and trimethoprim are capable of inhibiting distinct steps in the folic acid metabolism thus blocking the bacteria to obtain essential compounds required for their survival (Kapoor et al., 2017).

2.1.3. Chemical structure

Antibiotics within the same chemical structural class express similar patterns of effectiveness, toxicity and allergic potential (MicroDok, 2018). The β-lactam antibiotics, e.g. penicillins, amoxicillin and ampicillin, and the cephalosporins, e.g. cefalexin, all contain a beta-lactam ring in their structure and inhibit bacteria cell wall biosynthesis (Compound Interest 2014; Coates et al., 2011). Another example is the aminoglycosides, a family of over 20 antibiotics e.g. gentamicin, streptomycin and kanamycin, that all contain amino-sugar substructures and inhibit the synthesis of protein by the bacteria (Compound Interest 2014)

2.2. Antibiotic resistance mechanisms

The way the antibiotic is prescribed and used in the world urgently needs to change. WHO has identified antibiotic resistance as one of the three greatest threats of the 21st century to both human and animal health (WHO, 2014). Antibiotic resistance is rising to dangerously high levels where new mechanism of resistance are emerging and threatening the ability to treat the most common bacterial infectious diseases. It is estimated that 70% of the pathogenic bacteria are resistant to at least one of the known antibiotics (Garzón et al., 2020). The findings and development of new antibiotics have in the last couple of decades been scarce due to shortage of research on antibiotics (Munita and Arias, 2016). The chance of finding new antibiotics is also limited by that remaining effective antibiotics may be few to be discovered.

The principles of biology and evolution are unavoidable to the occurrence and rise of ARB (Sharma et al., 2016). Over millions of years of evolution sophisticated mechanisms of drug resistance have evolved from bacteria in order to avoid death by antimicrobial molecules, thus bacteria resistance towards antibiotics is a natural response (Munita and Arias, 2016; Awad et al., 2012). However, the widespread

misuse of antibiotics in both human and food-producing animals leads to accelerated spread of ARB (WHO, 2014). Bacteria can be intrinsically resistant to antibiotics due to the bacterial natural ability to resist the action of a certain antibiotic (Blair et al., 2015). This is a result of inherent functional or structural characteristics such as the absence of a susceptible target of a specific antibiotic. Antibiotic resistance may also be acquired via spontaneous mutations in chromosomal genes or by horizontal gene transfer from donor bacteria, phages or extrinsic additional DNA from an outside source (Sefton, 2002).

The mutational resistance occurs when susceptible bacterial cells develop mutations in genes that affect the antibiotic activity (Munita and Arias, 2016). This gives a selective survival advantage in the presence of the antibiotic as susceptible bacterial neighbors will be eliminated and the resistant bacteria can predominate the culture and further disseminate effectively (Walsh, 2003). The mutated resistant bacteria alter the antibiotic action via several mechanisms which can be divided into three groups: (i) enzymatic destruction or modification of the antibiotic, (ii) prevention of access to target, or (iii) replacement or modification of the antibiotic target.

2.2.1. Enzymatic destruction or modification of the antibiotic

Antibiotics can be destroyed through hydrolyzation by enzymes (Kapoor et al., 2017). One of the most common mechanisms of antibiotic resistance is the hydrolyzation of β-lactam antibiotics, e.g. penicillins, cephalosporins, monobactams and carbapenems by the enzymes β-lactamases (Bush and Mobashery, 1998; Blair et al., 2015). The β-lactamases hydrolyzes esters and amide bonds of the β-lactam ring thereby destroying the antibacterial activity. Shortly after penicillin became widely available, infections caused by a β-lactamase (penicillinase) producing bacteria, *Staphylococcus aureus,* became clinically relevant (Munita and Arias, 2016). In order to overcome the problem, improved β-lactam compounds were manufactured (e.g. ampicillin) with less susceptibility to penicillinase and with a wider spectrum of activity. However, with time, bacteria have become capable to hydrolyze the improved antibiotic. To date, more than 1,000 β-lactamases in a wide range of different bacteria are known to exist (Munita and Arias, 2016).

Enzymes that modify the antibiotic structure through chemical changes thus preventing it from binding to the target site, is another mechanism of acquired AR in both gram-positive and gram-negative bacteria (Blair et al., 2015). Antibiotics belonging to the group of aminoglycosides are large molecules with many exposed amide and hydroxyl groups. Aminoglycoside modifying enzymes target and modify the amide and hydroxyl groups, creating a steric hindrance that decrease the avidity of the antibiotic for its target (Munita and Arias, 2016).

2.2.2. Prevention of access to target

For the antibiotic to be able to reach its intracellular target and exert its antimicrobial effect in the gram-positive bacteria the cytoplasmic membrane needs to be penetrated (Blair et al., 2015; Munita and Arias, 2016). In the case of gram-negative bacteria, the antibiotics need to penetrate both the outer membrane and the inner, cytoplasmic membrane in order to reach its target. Hydrophilic antibiotics, e.g. β-lactams and tetracyclines, can diffuse through the gram-negative outer membrane by the water-filled diffusion channel protein called porins. Therefore, limiting the permeability and influx of antibiotics through the outer membrane porins have become gram-negatives' mechanism to prevent uptake of antibiotics. This is achieved by replacement of porins with more selective channels able to prevent access to certain antibiotics or by decrease the number of porins in the membrane (Kapoor et al., 2017; Blair et al., 2015). Bacteria with well-known porin meditated antibiotic resistance include *Pseudomonas aeruginosa* and *Escherichia coli* (Blair et al., 2015).

A second prevention of access to target in bacteria is by the active export of antibiotics out of the cell (Walsh, 2003). The export is controlled by the transmembrane proteins called efflux pumps. The efflux pumps are found in the cytoplasmic membrane of the bacteria and also in the outer membrane of the gram-negative bacteria and are used physiologically to pump out foreign toxic substrates and export of specific metabolites. There are various classes of efflux pumps in both gram-negative and gram-positive bacteria. The pumps may be substrate specific thus providing narrow specificity antibiotic resistance or have broad tolerance for a variety of structurally dissimilar compounds thus being associated with multidrug resistance (Webber and Piddock, 2003; Piddock, 2006).

The porin channels of the outer membrane in collaboration with efflux pumps give the gram-negative bacteria a selective advantage over their gram-positive counterparts in environments where antibiotics may be present thus making it more difficult to find antibiotics that target gram-negative bacteria (Tamber and Hancock, 2003; Imai et al., 2019).

2.2.3. Replacement or modification of the antibiotic target

Antibiotics bind to a target site on the bacteria thus preventing its normal activity (Walsh, 2003). The ability of modifying the antibiotics target site is a strategy that the pathogenic bacteria have to develop to avoid the action of the antibiotics. This can be achieved by complete replacement of the target site with evolving of new targets that express decreased sensitivity to the antibiotics but still accomplish similar biochemical functions of the native target (Munita and Arias, 2016). Gram-positive bacteria strains of *Staphylococcus aureus* and *Streptococcus pneumoniae* have developed resistance to β-lactam antibiotics using such strategy. Further strategies for target change involve chromosomal mutation in the genes encoding

the target site resulting in decreased affinity of the antibiotic to its target. Also, a modification of the target by addition of a chemical group on the target site can inhibit the binding of antibiotics.

2.2.4. Horizontal gene transfer

Horizontal gene transfer (HGT) is a mode of transfer of resistance frequently responsible for the development of ARB through which bacteria pass on their antibiotic resistant genes to susceptible non- resistant bacteria (MicroDok, 2018). Horizontally acquired genes can be exchanged through three main mechanism: (i) transformation, (ii) transduction, and (iii) conjugation (Thomas and Nielsen, 2005).

Transformation

DNA enters the surrounding environment upon release from bacterial cells that are disrupted or decomposed (Thomas and Nielsen, 2005). DNA can also be released from a living growing cell. The free genetic material is taken up by a bacterium and integrated into the recipient's cytoplasm (Holmes and Jobling, 1996). The incorporated DNA can benefit the bacterial cell with advantageous genes such as antibiotic resistant ones (MicroDok, 2018).

Transduction

Genes from a host bacterium are incorporated into a genome of a bacterial virus (bacteriophage) and the genetic material is further transferred to another bacteria (Griffiths et al., 2000). The genetic process of the recipient bacteria is taken over by the phage in order to produce more phages. Bacterial DNA that might contain antibiotic resistant genes may inadvertently be incorporated into the new phages. Upon cell lyses, the new phages bind to other bacteria and injects the antibiotic resistant genes into the cytoplasm where they further integrate into host chromosome (MicroDok, 2018).

Conjugation

Conjugation is the form of gene transfer that is mediated by direct contact between the donor and recipient bacteria through cell-to-cell junctions and pores (Thomas and Nielsen, 2005; Griffiths et al., 2000). The donor bacteria transfer one strand of plasmid DNA through the pore into the recipient bacteria where each single strand becomes double stranded again and integrates into the resident chromosome by recombination. The plasmids act as vehicles to share genetic information (Munita and Arias, 2016). Transposable elements are other important vehicles that can carry resistant genes and move within the genome and insert themselves into the host plasmids (Ferri et al., 2017). Further, integrons are elements that have site-specific integration systems capable of recruiting open reading frames in form of mobile gene cassettes thus enabling themselves to add new genes into the host

chromosome. These processes enable susceptible bacteria to acquire antibiotic resistant genes.

2.3. Antibiotics in livestock and food

Antibiotics were introduced in veterinary medicine in the 1950s. Today, they are mainly used to treat clinical cases, to control and prevent common diseases and as growth promoters (Marshall and Levy, 2011; Özdikmenli Tepeli and Demirel Zorba, 2018)

Earlier studies (Gupta et al., 2004; Normanno et al., 2007; Alexander et al., 2010; Özdikmenli Tepeli and Demirel Zorba, 2018) have demonstrated that consumers are exposed to ARB via food of animal origin e.g. beef, chicken and dairy products. The increasing antibiotic resistance in humans are linked with the therapeutic and nontherapeutic antibiotic use in livestock (Ferri et al., 2017). Transfer of ARB and resistant genes from farm to consumer via foods is quantitatively the most important mode of transmission (van den Bogaard and Stobberingh, 2000). Some bacteria found in food, expressing antibiotic resistance, have also been identified in humans (Marshall and Levy, 2011)

The most common reason for the use of antibiotics in dairy cows is intramammary infection (mastitis) (Pyörälä, 2009). Approximately 9 out of 100 cows in Sweden are treated for mastitis per year which is a reduction compared to 2001 with almost 50% (Växa 2019). The β-lactams are the most frequently used antibiotics within veterinary medicine in Sweden (Gustavsson, 2003). Milk from lactating cows treated with antibiotics are withdrawn during the statutory withdrawal period in order to let the antibiotic residues in the milk decrease below the maximum residue limit (Duse et al., 2013). The milk is not allowed to be delivered to the dairy during the withdrawal period (Gustavsson, 2003). However, if the farmer, deliberately or by accident, is not conforming to the withdrawal period and milk from dairy cows treated with antibiotics is deliverd to the dairy, it may increase the risk of development om ARB (Ndahetuye, 2019). Insufficient knowledge about the withdrawal periods is one of the most common causes of occurrence of antibiotic residues in milk in Sweden (Gustavsson, 2003). Insufficient identification of treated cows and failures due to hired staff are two other common causes. Further, milk and dairy products can also be contaminated by ARB through the production stages of the dairy product (Özdikmenli Tepeli and Demirel Zorba, 2018). Antibiotic residues in milk can also inhibit or impair the growth of starter cultures in dairy products such as cheese and yoghurt and thus cause a decrease in food quality and security (Brady and Katz, 1988; Ndahetuye, 2019). Therefore, rapid milk screening assays for detection of antibiotic residues are performed by dairy processors (Gustavsson, 2003). In Sweden, the Swedish

Food Agency requires that dairy cooperatives test milk from their farmers on regular basis to detect antibiotic residues (Livsmedelsverket, 2021).

2.4. Methods for determination of antibiotic resistant bacteria

EFSA and WHO have issued recommendations for laboratory testing for antibiotic resistant genes (Özdikmenli Tepeli and Demirel Zorba, 2018). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) describe the disk diffusion method. Microdilution assay and E-test are also some of the most common assays to determine antibiotic susceptibility among bacteria.

2.4.1. Kirby- Bauer disk diffusion method

A standardized procedure for the disk diffusion method was conducted in the 1960s by W.M.M Kirby and his colleague A.W. Bauer (Hudzicki, 2009). The Kirby-Bauer disk diffusion susceptibility test method determines the resistance or susceptibility of aerobic and facultative anaerobic bacteria to various antibiotics. Bacteria is grown on agar in the presence of antibiotic impregnated filter paper disks with a known concentration of the chosen compound (Biemer, 1973). The size of the zone of inhibition of growth around the disk is an indirect measure of the ability of that antibiotic to inhibit a certain type of bacteria (Hudzicki, 2009). Each antibiotic has a unique breakpoint zone indicating susceptibility to that antibiotic. The breakpoint zone diameter around the antibiotic disk observed from the analyzed bacteria is compared to the unique breakpoint zone to determine the susceptibility.

2.4.2. MALDI-TOF MS

Matrix-assisted laser desorption/ionization- time of flight mass spectrometry (MALDI-TOF MS) is a tool for microbial identification and diagnosis (Singhal et al., 2015). Maldi was introduced by Franz Hillenkamp and Michael Karas in the 1980s as a tool for analysis of large biomolecules (Jurinke et al., 2004). The sample for analysis is embedded with an organic compound called matrix. The sample and the matrix crystallize on drying and are irradiated with a laser beam causing desorption and ionization generating singly protonated ions. The ions mass-to-charge (m/z) ratios are calculated by measuring their time of flight required for the ions to travel the length of the flight tube and reach the detector (Singhal et al., 2015). Smaller ions will be traveling faster than larger ions (Croxatto et al., 2012). This creates a mass spectrum that is characterized by both the m/z and the number

of ions of a particular *m/z* that struck the detector. The mass spectra are analyzed and compared with stored profiles for species determination (Sandrin et al., 2013).

3. Experimental procedure

3.1. Products

This study included samples based on bovine milk products from a known dairy brand (Arla) available on the Swedish market. The products chosen were pasteurized milk (3% fat), fermented milk (unflavored yoghurt), fresh cheese (unflavored Keso) and ripened hard cheese (Herrgårdsost, 28% fat). All products were purchased from a local ICA store (ICA Maxi, Uppsala). Unpasteurized whole milk originating from a livestock farm in Sörmland, Sweden, was also included as a sample in this study. All samples were kept under refrigeration (4°C) until analyzes. Unpasteurized milk was used within two days from collection while the purchased samples were used until the expiring date of the product.

3.2. Antibiotics

In this study, nine antibiotics were chosen (Table 1) from seven classifications. The mechanism of action of the antibiotics were inhibition of cell wall biosynthesis and inhibition of protein biosynthesis. The antibiotic disks were obtained from Thermo Scientific Oxoid (Oxoid Ltd, Basingstoke, England) and stored in sealed cartridges at -20°C until use. In order to prevent condensation that could lead to rapid deterioration of some agents, the cartridges containing the antibiotic disks were temperate at room temperature (~22°C) before use.

Table 1. Antibiotics used in the study

Antibiotic	Abbreviation	Disc content (µg)	Classification
Ampicillin[1]	AMP	10	Beta-lactam penicillin
Chloramphenicol[2]	C	30	Amphenicol
Gentamicin[2]	CN	10	Aminoglycoside
Kanamycin[2]	K	30	Aminoglycoside
Streptomycin[2]	S	10	Aminoglycoside
Erythromycin[2]	E	15	Macrolide

Tetracycline[2]	TE	30	Tetracycline
Vancomycin[1]	VA	30	Glycopeptide
Clindamycin[2]	DA	2	Lincosamide

[1]: Inhibitors of cell wall biosynthesis (Compound Interest, 2014)
[2]: Inhibitors of protein biosynthesis (Compound Interest, 2014)

3.3. Material and methods

To investigate possible content of ARB in Swedish dairy products and raw milk the study was divided into three major steps: 1) Culturing and isolation of bacteria 2) antibiotic susceptibility test using disk diffusion methodology and 3) taxonomic identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Culturing was performed to isolate bacteria from the dairy product samples. The disk diffusion methodology was performed to determine susceptibility of the isolated bacteria towards the antibiotic whilst MALDI-TOF MS was performed in order to identify the taxonomy of the bacteria.

Equipment and facilities for this study were provided by Swedish University of Agricultural Sciences (SLU, Uppsala).

3.4. Culturing and isolation of bacteria

3.4.1. Media

Cultivation on agar plate was performed for all the dairy products using four different agar media. All the agar plates were prepared according to the manufacturer's instructions and stored at 4°C until usage.

De Man, Rogosa and Sharpe (MRS) agar stimulates growth of *Lactobacillus*. However, MRS agar also stimulates growth of other lactic acid bacteria species of the genera *Streptococcus*, *Pediococcus*, *Leuconostoc* and *Lactococci* (Sigma-Aldrich[1] MRS 2020; Oxoid[1] - Product Detail MRS, 2020). The MRS agar plates used for fresh cheese and fermented milk were incubated anaerobically at 42°C for growth of thermophilic LAB whilst MRS agar plates for ripened hard cheese, unpasteurized and pasteurized milk were incubated anaerobically at 30°C for growth of mesophilic LAB. All MRS agar plates were incubated for approximately 48 hours.

M17 agar with 10% lactose solution (w/v) stimulates growth of *Streptococcus*, but less extensive *Lactobacillus* in yoghurt and other dairy products (Oxoid[2] - Product Detail M17, 2020; Difco and BBL 2003). M17 agar plates were incubated aerobically at 30°C for approximately 48 hours. The M17 agar plates were not used

for ripened hard cheese samples due to low survival rate of *Streptococcus* after the ripening process (Yale and Marquardt, 1940).

Mannitol Salt Phenol Red agar (MSPR) mainly stimulates the growth of *Staphylococcus* and is recommended for the detection of these bacteria in milk and food (Oxoid[3] - Product Detail, MSPR 2020). The MSPR agar plates were incubated aerobically at 37°C for approximately 24 hours.

Finally, Violet Red Bile Dextrose agar (VRBD) were used for detection of coliforms. Coliform bacteria are commonly used as an indicator organism for unsanitary conditions in pasteurized dairy products (Martin et al., 2016). The VRBD agar plates were incubated aerobically at 37°C for approximately 24 hours.

Agar for MRS and VRBD was obtained from Merck KGaA (Merck KGaA, Darmstadt, Germany) whilst M17 and MSPR agar were obtained from Sigma-Aldrich (Sigma-Aldrich, Co., St Louise, MO, USA).

3.4.2. Preparation of buffer solution

Peptone water buffer containing 10 g peptone (Oxoid Ltd, Basingstoke, Hampshire, England) and 5 g sodium chloride dissolved in 1 L distilled water was prepared. Saline solution buffer containing 9 g sodium chloride was dissolved in 1 L distilled water. The buffers were sterilized by autoclaving at 125°C for 15 minutes. Also, a glycerol stock containing 100 mL 99,5% bi-distilled glycerol and 100 mL distilled water was prepared and autoclaved at 125°C for 15 minutes. All chemicals and equipment, if not indicated differently, were obtained from VWR Chemicals (VWR International, Leuven, Belgium).

3.4.3. Isolation of bacteria

Both ripened hard cheese and fresh cheese samples were added to 0.1% peptone water (w/v) buffer at the ratio of 1:10 (w/v) and homogenized in a stomacher (Seward Stomacher, 400-circulator, England) at 230 rpm for 2 minutes. Unpasteurized-, pasteurized- and fermented milk samples were added to saline solution buffer at the ratio of 1:10 (w/v). From the homogenized samples 1 mL of aliquot was used for a dilution series. Serial dilutions were made from all five dairy product samples with a dilution factor of 10-fold, until 10^{-3}.

The experiment was performed using two different plate methods, track dilution and traditional plate method. Both methods were performed on M17, MRS, MSPR and VRBD plates for all five dairy product samples, respectively, with an exception for M17 that was not used for ripened hard cheese.

Track dilution was performed from the dilution series following the method from Jett et al. (1997). For each 10-fold dilution 10 µL was added onto one agar plate. The agar plate was tipped onto its side (at a 45°-90° angle) to allow the aliquot to

migrate in parallel tracks across the agar surface to the opposite side of the agar plate. Each dilution was analyzed in triplicates.

In case where colony yield from track dilution was low, traditional plate method was performed using 100 µL non- diluted sample taken directly from the product package of pasteurized milk, fermented milk and unpasteurized milk respectively. For the ripened hard cheese and fresh cheese 100 µL were taken from the homogenized mixture. Samples were added onto the agar and spread over the surface using a sterile L-shaped spreader. Each dairy product sample was analyzed in triplicates. Incubation temperature and time were adjusted according to the isolated targeted bacteria.

After the incubation period, single colony of isolated bacteria was inoculated onto new agar plates by using quartered plate area and streaking technique in order to obtain pure culture. Agar plates were incubated as previously stated. After the incubation period the purification step was repeated once more.

Further, a single colony of each isolated bacteria was spread on quartered plate area and incubated for cultivation. After cultivation, all colonies of each isolated bacteria were transferred to a screw top micro tube (2 mL) with 1 mL of 50% glycerol, vortexed, and stored in a freezer at -80°C until further analysis.

The purification step was performed on all growth media agar plates with samples from each dairy product respectively. Growth media that showed no bacteria growth after purification step were not used further in the study.

3.5. Antimicrobial susceptibility test

The antimicrobial susceptibility test (AST) was performed following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) disk diffusion method protocol, version 8.0, 2020 (EUCAST 2020).

3.5.1. Media

Mueller Hinton (MH) agar was prepared according to the manufacture's (Sigma-Aldrich, Co., St Louise, MO, USA) instructions and stored at 4°C until usage. MH agar is a non-selective, non-differential medium that is commonly used for susceptibility testing of pathogens (Sigma-Aldrich[2] 2021, Mueller-Hinton Agar 2021; Aryal 2020). The MH agar plates were incubated aerobically at 30°C for approximately 24 hours. MRS, M17 and VRBD were also used as cultivating growth media for the AST and incubated according to the time and temperature previously stated.

3.5.2. Preparation of turbidity standard

In order to perform AST using EUCAST disk diffusion method, a cell suspension of organism's equivalent to a 0.5 McFarland turbidity standard was prepared. A 0.5 McFarland turbidity standard provides an optical density corresponding to the density of a bacterial suspension of approximately 1.5x 10^8 colony forming units (CFU/mL) (EUCAST, 2020).

The 0.5 McFarland turbidity standard consisted of 0.5 mL of 0.048 mol/L barium chloride and 99.5 mL of 0.18 mol/L sulfuric acid, prepared according to the protocol of EUCAST 2020 and stored dark at room temperature (25°C) until usage. Chemicals were obtained from Merck KGaA (Darmstadt, Germany).

3.5.3. Disk diffusion method

Bacteria from the stock stored at -80ºC was inoculated onto new agar plates for re-cultivation. Agar plates for re-cultivation corresponded to the growth media once used for cultivation. Bacteria were also inoculated onto MH agar plates. Agar plates were incubated after inoculation. The following steps were performed for all stored isolated bacteria.

After incubation, the re-cultivated bacteria were inoculated into a sterilized test tube with 1 mL saline solution. The suspension of the bacterial cells was made to the density of a 0.5 McFarland turbidity standard. By holding the test tube suspension of bacterial cells next to the beaker of 0.5 McFarland turbidity standard in front of a light, the density of the suspension was determined by visual reading. Additional saline solution was added to dilute the suspension when the density was too high whilst additional bacteria were inoculated into the suspension when the density was insufficient.

Upon correct density, the suspension of the bacterial cells was spread onto six new agar plates for cultivation by using a sterile cotton swab on a wooden applicator. The inoculum was evenly spread over the entire surface of the agar by swabbing in three directions, ensuring no gaps between the streaks.

Further, onto each inoculated agar plate three different antibiotic disks were firmly applied to the surface of the agar. All nine antibiotics were analyzed in duplicates. Agar plates were then carefully inverted to ensure that antibiotic disks were not falling off the agar surface. All agar plates were incubated within 15 minutes of disk application.

After incubation, the susceptibility of the bacteria toward the antibiotics was determined through measurement of the diameters of the inhibition zone to the nearest whole millimeter using a ruler. The zone edges were read as the point showing no growth viewed from the back of the agar plate.

3.6. MALDI-TOF MS

The isolated antibiotic resistant bacteria were taxonomically identified using MALDI-TOF MS (MALDI Biotyper, Micoflex, Bruker Daltonik GmbH) with direct transfer-formic acid method.

A fresh cultivation of bacteria was performed from stock bacteria at -80°C added onto new agar plates and incubated. After incubation, a single colony of freshly cultured colonies was picked using a toothpick and smeared onto a spot on a Maldi target plate (MTP). Onto the surface where the bacterial colony was smeared, 1µL of 70% formic acid was added and allowed to air dry. Further, a matrix of 1µL of saturated α-cyano-4-hydroxycinnamic acid (HCCA) mixed in 50% acetonitrile-2.5% trifluoroacetic acid was added onto the surface and air dried at room temperature (20-25ºC). The MTP was loaded to the port of MALDI-TOF MS and the bacterial identification analysis was performed. In total, 48 bacteria could be identified by using MALDI-TOF MS. Each identified bacterium was given an isolate identification number (isolate-id) based on the product and growth media used for the isolation. Bacteria identified from unpasteurized milk were given isolate-id B1-6, C1-6 and D1-6, fresh cheese E1-6, ripened hard cheese F1-6, pasteurized milk G1-6 and fermented milk H1-6 and I1-6.

3.6.1. Data processing

Antimicrobial susceptibility testing breakpoint tables for zone diameter (ZD) were collected from literature (Table 2). All identified bacteria were classified according to the measured ZD and the corresponding susceptibility breakpoint. However, for bacteria species where insufficient or no susceptibility breakpoint data could be found from the literature, ZD susceptibility breakpoints data were used from bacteria within the same genus classification.

Table 2. Sources for antimicrobial susceptibility testing breakpoint tables for zone diameters

Bernal-Rosas et al., 2015
Charteris et al., 1998
Eady et al., 2000
EUCAST 2021
OXOID 2013

4. Results

The antibiotics were analyzed in duplicates and the ZD were measured (see Appendix 2). Average ZD were calculated in millimeter (mm) and compared with ZD from susceptibility breakpoint tables. The results were expressed according to the clinical categories susceptible (S) intermediate (I) and resistant (R). Bacteria that express susceptibility are inhibited by the concentration of the antibiotic. The antibiotic is associated with a high likelihood of therapeutic success upon a susceptible result (EUCAST 2021). Intermediate bacteria are inhibited by the concentration of the antibiotic. However, the antibiotic is associated with uncertain therapeutic effect upon an intermediate result. Resistant bacteria are not, or partly, inhibited by the concentration of the antibiotic. The antibiotic is associated with high likelihood of therapeutic failure upon a resistant result. For antibiotics and bacteria where no susceptibility breakpoint tables data for ZD were available, the results were expressed as no data available (N/A).

From the 48 bacteria analyzed with MALDI-TOF MS 14 bacteria species were identified (Table 3). The number of times a bacteria species was isolated differ depending on the product and growth media.

Table 3. Identified bacteria by MALDI-TOF MS from the unpasteurized milk and dairy products

Taxonomy	Unpasteurized milk	Pasteurized milk	Fresh cheese	Ripened hard cheese	Fermented milk
Bacillus licheniformis		n=1			
Kocuria varians		n=5			
Lactobacillus paracasei	n=2			n=6	
Lactobacillus delbrueckii					n=5
Micrococcus luteus					n=1
Pediococcus pentosaceus	n=4				
Pseudomonas brenneri			n=6		
Pseudomonas fulva	n=6				n=1
Raoultella ornithinolytica	n=5				
Raoultella planticola					n=1
Raoultella terrigena					n=1
Staphylococcus hominis		n=1			n=1
Stenotrophomonas maltophilia	n=1				
Streptococcus salivarius					n=1

Number of bacteria isolated in the product (n)

4.1. Unpasteurized milk

The bacteria present in unpasteurized milk is displayed in Table 4. In total, five bacteria species (*Raoultella ornithinolytica, Stenotrophomonas maltophilia, Pseudomonas fulva, Pediococcus pentosaceus, Lactobacillus paracasei*) were identified. Bacteria with isolate-id B1-6 were isolated on VRBD agar plate and re-cultivated on MH agar with antibiotic disks. Bacteria with isolate-id C1-6 were isolated on M17 agar plate and re-cultivated on MH agar plate with antibiotic disks. The MRS agar plate was used for both isolation of bacteria and re-cultivation with antibiotic disks for bacteria with isolate-id D1-6.

4.1.1. *Raoultella ornithinolytica*

Full susceptibility ZD breakpoint tables data were available for *Raoultella ornithinolytica* (*R. ornithinolytica)* and the antibiotics. *R. ornithinolytica* expressed resistance towards five antibiotics, ampicillin (AMP), erythromycin (E), streptomycin (S), vancomycin (VA), clindamycin (DA) and susceptibility for four antibiotics, chloramphenicol (C), gentamicin (CN), kanamycin (K), tetracycline (TE). *R. ornithinolytica* indicated resistance towards two antibiotic mechanisms of action: inhibition of cell wall biosynthesis and inhibition of protein biosynthesis.

4.1.2. *Stenotrophomonas maltophilia*

There were no susceptibility ZD breakpoint tables data available for *Stenotrophomonas maltophilia* (*S. maltophilia)*, bacteria from the same genus, or the antibiotics. However, *S. maltophilia* expressed full resistance towards three antibiotics (E, VA, DA). *S. maltophilia* indicated resistance towards two antibiotic mechanisms of action: inhibition of cell wall biosynthesis and inhibition of protein biosynthesis.

4.1.3. *Pseudomonas fulva*

Specified susceptibility ZD breakpoint tables data were not available for *Pseudomonas fulva* (*P. fulva)*. The breakpoint data were instead collected for *Pseudomonas aeruginosa (P. aeruginosa)*, a bacterium from the same genus. Two of the antibiotics (K, S) had no available ZD breakpoint data. *P. fulva* expressed resistance towards four antibiotics (AMP, E, VA, DA), intermediate for one antibiotic (C) and susceptibility towards two antibiotics (CN, TE). *P. fulva* indicated resistance towards two antibiotic mechanisms of action: inhibition of cell wall biosynthesis and inhibition of protein biosynthesis.

4.1.4. *Pediococcus pentosaceus*

Full susceptibility ZD breakpoint tables data were available for *Pediococcus pentosaceus* (*P. pentosaceus)* and the antibiotics. *P. pentosaceus* expressed resistance towards four antibiotics (CN, K, S, VA) and susceptibility towards five antibiotics (AMP, C, E, TE, DA). *P. pentosaceus* indicated resistance towards two antibiotic mechanisms of action: inhibition of cell wall biosynthesis and inhibition of protein biosynthesis.

4.1.5. *Lactobacillus paracasei*

Full susceptibility ZD breakpoint tables data were available for *Lactobacillus paracasei* (*L. paracasei)* and the antibiotics. *L. paracasei* expressed resistance towards four antibiotics (CN, K, S, VA), intermediate towards one antibiotic (TE) and susceptibility towards four antibiotics (AMP, C, E, DA). *L. paracasei* indicated resistance towards two antibiotic mechanisms of action: inhibition of cell wall biosynthesis and inhibition of protein biosynthesis.

Table 4. Antibiotic susceptibility for R. ornithinolytica (n=5), S. maltophilia (n=1), P. fulva (n=6), P. pentosaceus (n=4) and L. paracasei (n=2) isolated in unpasteurized milk

Isolate ID	Taxonomy	AMP	C	CN	E	K	S	TE	VA	DA
		ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S
B1	*R. ornithinolytica*	11 | R	26 | S	18 | S	0 | R	20 | S	11 | R	25 | S	0 | R	0 | R
B2	*R. ornithinolytica*	10 | R	25 | S	18 | S	0 | R	20 | S	17 | S	25 | S	0 | R	0 | R
B3	*S. maltophilia*	12 | N/A	26 | N/A	19 | N/A	0 | R	21 | N/A	17 | N/A	26 | N/A	0 | R	0 | R
B4	*R. ornithinolytica*	11 | R	25 | S	20 | S	0 | R	21 | S	18 | S	26 | S	0 | R	0 | R
B5	*R. ornithinolytica*	10 | R	26 | S	19 | S	0 | R	21 | S	18 | S	25 | S	0 | R	0 | R
B6	*R. ornithinolytica*	11 | R	27 | S	20 | S	0 | R	22 | S	18 | S	25 | S	0 | R	0 | R
C1	*P. fulva*	0 | R	14 | I	19 | S	0 | R	25 | N/A	13 | N/A	25 | S	0 | R	0 | R
C2	*P. fulva*	0 | R	14 | I	20 | S	0 | R	25 | N/A	12 | N/A	25 | S	0 | R	0 | R
C3	*P. fulva*	0 | R	13 | I	20 | S	0 | R	24 | N/A	13 | N/A	26 | S	0 | R	0 | R
C4	*P. fulva*	0 | R	14 | I	20 | S	0 | R	25 | N/A	12 | N/A	25 | S	0 | R	0 | R
C5	*P. fulva*	0 | R	14 | I	20 | S	0 | R	25 | N/A	13 | N/A	25 | S	0 | R	0 | R
C6	*P. fulva*	0 | R	13 | I	20 | S	0 | R	25 | N/A	12 | N/A	25 | S	0 | R	0 | R
D1	*P. pentosaceus*	33 | S	35 | S	0 | R	35 | S	0 | R	0 | R	40 | S	0 | R	26 | S
D2	*P. pentosaceus*	20 | S	28 | S	0 | R	25 | S	0 | R	0 | R	19 | S	0 | R	26 | S
D3	*P. pentosaceus*	25 | S	34 | S	0 | R	34 | S	0 | R	0 | R	29 | S	0 | R	28 | S
D4	*L. paracasei*	20 | S	25 | S	0 | R	25 | S	0 | R	0 | R	18 | I	0 | R	26 | S
D5	*L. paracasei*	23 | S	26 | S	0 | R	26 | S	0 | R	0 | R	17 | I	0 | R	26 | S
D6	*P. pentosaceus*	20 | S	25 | S	0 | R	25 | S	0 | R	0 | R	20 | S	0 | R	26 | S

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA), Zone diameter (mm) (ZD), Resistant (R), Intermediate (I), Susceptible (S), No data available (N/A)

4.2. Fresh cheese

The bacteria present in fresh cheese are displayed in Table 5. A single bacteria species (*Pseudomonas brenneri)* was identified. Bacteria with isolate-id E1-6 were isolated on M17 agar plate and re-cultivated on MH agar plate with antibiotic disks.

4.2.1. *Pseudomonas brenneri*

Specified susceptibility ZD breakpoint tables data were not available for *Pseudomonas brenneri* (*P. brenneri)*. The ZD breakpoint data were collected from *P. aeruginosa*, a bacterium from the same genus. Two of the antibiotics (K, S) had no available ZD breakpoint data. *P. brenneri* expressed resistance towards five antibiotics (AMP, C, E, VA, DA) and susceptibility towards two antibiotics (CN, TE). *P. brenneri* indicated resistance towards two antibiotic mechanisms of action: inhibition of cell wall biosynthesis and inhibition of protein biosynthesis.

Table 5. Antibiotic susceptibility for P. brenneri (n=6) isolated in fresh cheese

Isolate ID	Taxonomy	AMP	C	CN	E	K	S	TE	VA	DA
		ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S
E1	*P. Brenneri*	0 | R	10 | R	23 | S	0 | R	22 | N/A	15 | N/A	33 | S	0 | R	0 | R
E2	*P. Brenneri*	0 | R	8 | R	24 | S	0 | R	29 | N/A	16 | N/A	34 | S	0 | R	0 | R
E3	*P. Brenneri*	0 | R	7 | R	23 | S	0 | R	28 | N/A	17 | N/A	32 | S	0 | R	0 | R
E4	*P. Brenneri*	0 | R	8 | R	20 | S	0 | R	29 | N/A	18 | N/A	31 | S	0 | R	0 | R
E5	*P. Brenneri*	0 | R	9 | R	21 | S	0 | R	24 | N/A	15 | N/A	37 | S	0 | R	0 | R
E6	*P. Brenneri*	0 | R	8 | R	24 | S	0 | R	29 | N/A	17 | N/A	32 | S	0 | R	0 | R

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA), Zone diameter (mm) (ZD), Resistant (R), Intermediate (I), Susceptible (S), No data available (N/A)

4.3. Ripened hard cheese

The bacteria present in ripened hard cheese is displayed in Table 6. A single bacteria species (*Lactobacillus paracasei*) was identified. The MRS agar plate was used for both isolation of bacteria and re-cultivation with antibiotic disks for bacteria with isolate-id F1-6.

4.3.1. *Lactobacillus paracasei*

Full susceptibility ZD breakpoint tables data were available for *Lactobacillus paracasei* (*L. paracasei*) and the antibiotics. *L. paracasei* expressed resistance towards four antibiotics (CN, K, S, VA) and susceptibility towards five antibiotics (AMP, C, E, TE, DA). *L. paracasei* indicated resistance towards two antibiotic mechanisms of action: inhibition of cell wall biosynthesis and inhibition of protein biosynthesis.

Table 6. Antibiotic susceptibility for L. paracasei (n=6) isolated in ripened hard cheese

Isolate ID	Taxonomy	AMP	C	CN	E	K	S	TE	VA	DA
		ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S
F1	L. *Paracasei*	33 | S	37 | S	0 | R	35 | S	0 | R	0 | R	36 | S	0 | R	28 | S
F2	L. *Paracasei*	30 | S	31 | S	0 | R	34 | S	0 | R	0 | R	35 | S	0 | R	28 | S
F3	L. *Paracasei*	28 | S	30 | S	0 | R	32 | S	0 | R	0 | R	35 | S	0 | R	26 | S
F4	L. *Paracasei*	26 | S	29 | S	0 | R	32 | S	0 | R	0 | R	34 | S	0 | R	25 | S
F5	L. *Paracasei*	31 | S	33 | S	0 | R	34 | S	0 | R	0 | R	35 | S	0 | R	25 | S
F6	L. *Paracasei*	35 | S	35 | S	0 | R	33 | S	0 | R	0 | R	35 | S	0 | R	24 | S

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA), Zone diameter (mm) (ZD), Resistant (R), Intermediate (I), Susceptible (S), No data available (N/A)

4.4. Pasteurized milk

The bacteria present in pasteurized milk is displayed in Table 7. Three bacteria species (*Bacillus licheniformis, Kocuria varians, Staphylococcus hominis)* were identified. Bacteria with isolate-id G1-6 were isolated on M17 agar plate and re-cultivated on MH agar plate with antibiotic disks.

4.4.1. *Bacillus licheniformis*

The availability of susceptibility ZD breakpoint tables data for *Bacillus licheniformis* (*B. licheniformis*), bacteria from the same genus and for the antibiotics, was limited. However, *B. licheniformis* expressed susceptibility towards three antibiotics (E, VA, DA). No additional ZD breakpoint data were available for remaining antibiotics. *B. licheniformis* indicated no resistance.

4.4.2. *Kocuria varians*

There were no susceptibility ZD breakpoint tables data available for *Kocuria varians* (*K. varians)*, bacteria from the same genus or the antibiotics. However, two isolated bacteria identified as *K. varians* (G5 and G6) expressed full resistance towards one antibiotic (DA). *K. varians* indicated resistance towards one antibiotic mechanism of action: inhibition of protein biosynthesis.

4.4.3. *Staphylococcus hominis*

The availability of susceptibility ZD breakpoint tables data for *Staphylococcus hominis* (*S. hominis),* bacteria from the same genus and for the antibiotics was limited. Two of the antibiotics (S, VA) had no available ZD breakpoint data. *S. hominis* expressed resistance towards four antibiotics (AMP, CN, K, DA) and susceptibility towards three antibiotics (C, E, TE). *S. hominis* indicated resistance towards two antibiotic mechanism of action: inhibition of cell wall biosynthesis and inhibition of protein biosynthesis.

Table 7. Antibiotic susceptibility for S. hominis (n=1) and B. licheniformis (n=1) isolated in pasteurized milk. No data available for K. varians (n=4)

Isolate ID	Taxonomy	AMP	C	CN	E	K	S	TE	VA	DA
		ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S
G1	K. *varians*	61 | N/A	39 | N/A	23 | N/A	50 | N/A	19 | N/A	22 | N/A	45 | N/A	24 | N/A	40 | N/A
G2	K. *varians*	63 | N/A	39 | N/A	22 | N/A	50 | N/A	21 | N/A	24 | N/A	47 | N/A	25 | N/A	41 | N/A
G3	S. *hominis*	13 | R	26 | S	20 | R	31 | S	13 | R	9 | N/A	42 | S	24 | N/A	0 | R
G4	B. *licheniformis*	65 | N/A	42 | N/A	25 | N/A	52 | S	18 | N/A	21 | N/A	47 | N/A	24 | S	42 | S
G5	K. *varians*	15 | N/A	31 | N/A	20 | N/A	30 | N/A	15 | N/A	8 | N/A	41 | N/A	22 | N/A	0 | R
G6	K. *varians*	13 | N/A	29 | N/A	18 | N/A	29 | N/A	14 | N/A	8 | N/A	41 | N/A	22 | N/A	0 | R

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA), Zone diameter (mm) (ZD), Resistant (R), Intermediate (I), Susceptible (S), No data available (N/A)

4.5. Fermented milk

The bacteria present in fermented milk is displayed in Table 8. In total seven bacteria species (*Streptococcus salivarius, Raoultella terrigena, Pseudomonas fulva, Micrococcus luteus, Staphylococcus hominis, Raoultella planticola, Lactobacillus delbrueckii*) were identified. Bacteria with isolate-id H1-6 were isolated on M17 agar plate and re-cultivated on M17 agar plate with antibiotic disks. The MRS agar plate was used for both isolation of bacteria and re-cultivation with antibiotic disks for bacteria with isolate-id I1-6.

4.5.1. *Streptococcus salivarius*

The availability of susceptibility ZD breakpoint tables data for *Streptococcus salivarius* (*S. salivarius),* bacteria from the same genus and for the antibiotics was limited. Four of the antibiotics (AMP, CN, K, S) had no available ZD breakpoint data. *S. salivarius* expressed susceptibility towards five antibiotics (C, E, TE, VA, DA). *S. salivarius* indicated no resistance.

4.5.2. *Raoultella terrigena*

The availability of susceptibility ZD breakpoint tables data for *Raoultella terrigena* (*R. terrigena),* bacteria from the same genus and for the antibiotics was limited. Three of the antibiotics (E, VA, DA,) had no available ZD breakpoint data. *R. terrigena* expressed resistance towards three antibiotics (CN, K, S) and susceptibility towards three antibiotics (AMP, C, TE,). *R. terrigena* indicated resistance towards one antibiotic mechanism of action: inhibition of protein biosynthesis.

4.5.3. *Pseudomonas fulva*

Specified susceptibility ZD breakpoint tables data were not available for *Pseudomonas fulva* (*P. fulva)*. The breakpoint data were collected from *P. aeruginosa),* a bacterium from the same genus. Three of the antibiotics (K, S, VA) had no available ZD breakpoint data. *P. fulva* expressed resistance towards one antibiotic (CN) and susceptibility towards five antibiotics (AMP, C, E, TE, DA). *P. fulva* indicated resistance towards one antibiotic mechanism of action: inhibition of protein biosynthesis.

4.5.4. *Micrococcus luteus*

There were no susceptibility ZD breakpoint tables data available for *Micrococcus luteus* (*M. luteus*), bacteria from the same genus or the antibiotics.

4.5.5. *Staphylococcus hominis*

The availability of susceptibility ZD breakpoint tables data for *Staphylococcus hominis* (*S. hominis),* bacteria from the same genus and for the antibiotics was limited. Two of the antibiotics (S, VA) had no available ZD breakpoint data. *S. hominis* expressed resistance towards two antibiotics (CN, K,) and susceptibility towards five antibiotics (AMP, C, E, TE, DA). *S. hominis* indicated resistance towards one antibiotic mechanism of action: inhibition of protein biosynthesis.

4.5.6. *Raoultella planticola*

The availability of susceptibility ZD breakpoint tables data for *Raoultella planticola* (*R. planticola),* bacteria from the same genus and for the antibiotics was limited. Three of the antibiotics (E, VA, DA) had no available ZD breakpoint data. *R. planticola* expressed resistance towards three antibiotics (CN, K, S) and susceptibility towards three antibiotics (AMP, C, TE,). *R. planticola* indicated resistance towards one antibiotic mechanism of action: inhibition of protein biosynthesis.

4.5.7. *Lactobacillus delbrueckii*

Full susceptibility ZD breakpoint tables data were available for *Lactobacillus delbrueckii* (*L. delbrueckii)* and the antibiotics. However, one isolated bacterium identified as *L. delbrueckii* (I6) had insufficient amount of growth due to material issues and was therefore not included in the results. *L. delbrueckii* expressed a variation in susceptibility among the isolated bacteria. Two of the *L. delbrueckii* isolates (I1, I3) expressed susceptibility towards all 9 antibiotics. However, *L. delbrueckii* bacteria I2 and I4 expressed resistance towards three antibiotics (CN, K, S) and susceptibility towards six antibiotics (AMP, C, E, TE, VA, DA). Finally,

L. delbrueckii bacteria I5 expressed intermediate towards one antibiotic (K) and susceptibility towards eight antibiotics (AMP, C, CN, E, S, TE, VA, DA). The *L. delbrueckii* indicated resistance towards one antibiotic mechanism of action: inhibition of protein biosynthesis.

Table 8. Antibiotic susceptibility for S. salavarius (n=1), R. terrigena (n=1), P. fulva (n=1), S. hominis (n=1), R. planticola (n=1) and L. delbrueckii (n=5) isolated in fermented milk. No data available for M. luteus (n=1)

Isolate ID	Taxonomy	AMP	C	CN	E	K	S	TE	VA	DA
		ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S	ZD | R/I/S
H1	*S. salivarius*	42 | N/A	40 | S	26 | N/A	43 | S	24 | N/A	23 | N/A	44 | S	25 | S	25 | S
H2	*R. terrigena*	50 | S	40 | S	15 | R	47 | N/A	9 | R	12 | R	47 | S	27 | N/A	50 | N/A
H3	*P. fulva*	40 | S	34 | S	13 | R	38 | S	10 | N/A	12 | N/A	40 | S	24 | N/A	25 | S
H4	*M. luteus*	50 | N/A	37 | N/A	21 | N/A	46 | N/A	11 | N/A	11 | N/A	44 | N/A	30 | N/A	46 | N/A
H5	*S. hominis*	42 | S	36 | S	15 | R	42 | S	10 | R	11 | N/A	40 | S	26 | N/A	45 | S
H6	*R. planticola*	49 | S	44 | S	16 | R	49 | N/A	10 | R	12 | R	48 | S	29 | N/A	53 | N/A
I1	L. *delbrueckii*	60 | S	41 | S	19 | S	45 | S	23 | S	25 | S	48 | S	33 | S	50 | S
I2	L. *delbrueckii*	54 | S	45 | S	12 | R	44 | S	11 | R	10 | R	44 | S	35 | S	44 | S
I3	L. *delbrueckii*	55 | S	45 | S	19 | S	42 | S	22 | S	22 | S	44 | S	35 | S	42 | S
I4	L. *delbrueckii*	50 | S	42 | S	12 | R	41 | S	0 | R	10 | R	41 | S	33 | S	41 | S
I5	L. *delbrueckii*	53 | S	40 | S	17 | S	41 | S	17 | I	17 | S	40 | S	30 | S	40 | S
I6	L. *delbrueckii*	N/A |	N/A |	N/A |	N/A |	N/A |	N/A |	N/A |	N/A |	N/A |

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA), Zone diameter (mm) (ZD), Resistant (R), Intermediate (I), Susceptible (S), No data available (N/A)

4.6. Antibiotic resistant bacteria

From the 14 identified bacteria species from the five milk and dairy samples 11 bacteria species expressed resistance towards at least one antibiotic (Table 9). Bacteria species *P. brenneri, P. fulva, and R. ornithinolytica* expressed the highest frequency of resistance towards a total of five antibiotics, respectively. Gentamicin was the most common antibiotic to which bacteria expressed resistance. In total, seven bacteria species expressed resistance towards this compound. Six bacteria species expressed resistance towards streptomycin, kanamycin, vancomycin and clindamycin respectively. Table 10 displays the frequency of resistance and number of single bacteria species in the milk and each dairy product.

Table 9. Antibiotic resistance distribution among the identified bacteria towards the antibiotics

Taxonomy	AMP	C	CN	E	K	S	TE	VA	DA	Total[1]
B. licheniformis										0
K. varians									R	1
L. paracasei			R		R	R		R		4
L. delbrueckii			R		R	R				3
M. luteus										N/A
P. pentosaceus			R		R	R		R		4
P. brenneri	R	R		R				R	R	5
P. fulva	R		R	R				R	R	5
R. ornithinolytica	R			R		R		R	R	5
R. planticola			R		R	R				3
R. terrigena			R		R	R				3
S. hominis	R		R		R				R	4
S. maltophilia				R				R	R	3
S. salivarius										0
Total[2]	4	1	7	4	6	6	0	6	6	-

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA), Resistant (R), No data available (N/A)

[1]: Total number of antibiotics the bacteria express resistance towards

[2]: Total number of bacteria that expressed resistance towards specific antibiotic

Table 10 Distribution of antibiotic resistant bacteria among the unpasteurized milk and dairy products

Taxonomy	Unpasteurized milk	Pasteurized milk	Fresh cheese	Ripened hard cheese	Fermented milk	Total[1]
Bacillus licheniformis						0
Kocuria varians		R				1
Lactobacillus paracasei	R			R		2
Lactobacillus delbrueckii					R	1
Micrococcus luteus						N/A
Pediococcus pentosaceus	R					1
Pseudomonas brenneri			R			1
Pseudomonas fulva	R				R	2
Raoultella ornithinolytica	R					1
Raoultella planticola					R	1
Raoultella terrigena					R	1
Staphylococcus hominis		R			R	2
Stenotrophomonas maltophilia	R					1
Streptococcus salivarius						0
Total[2]	5	2	1	1	5	-

Abbreviations: Resistant (R), No data available (N/A),

[1]: Total number of occasions bacteria occur as resistant in unpasteurized milk and/or dairy products

[2]: Total number of resistant bacteria species isolated in the unpasteurized milk or dairy product

5. Discussion

From the results of this study, it can be concluded that ARB was detected in unpasteurized milk and the four dairy products. As environmental and hygienic conditions at the farms and dairy plants are unknown, it is not possible to determine how these factors may have influenced the products ARB exposure and the ARB growth. However, the unpasteurized and fermented milk indicated the largest diversity of isolated ARB species. Five isolated species from each sample expressed resistance (Table 10). Two ARB species were isolated from the pasteurized milk. From fresh cheese and ripened hard cheese one antibiotic resistant bacteria specie was isolated. Antibiotic resistant LAB was isolated in unpasteurized milk, ripened hard cheese and fermented milk. No LAB was isolated from pasteurized milk and fresh cheese. The LAB may not have survived the pasteurization of the milk used for these products. No starter cultures used in the manufacturing may be a further reason why no LAB was detected in fresh cheese. Özdikmenli Tepeli and Demirel Zorba (2018) stated that milk and dairy products are the most incriminated foods acting as primary transmitters of ARB and antibiotic resistant genes to humans. Taking that statement in consideration, the ARB findings from this study indicate that the situation might be alarming. Especially since the antibiotics used in this study belong to the most important antibiotic classes used in veterinary and human medicine (Campedelli et al., 2019).

Gentamicin was the protein biosynthesis inhibiting antibiotic associated with the highest diversity of bacteria species expressing resistance. Seven different bacteria species expressed resistance towards gentamicin. Kanamycin, streptomycin and clindamycin resistance was expressed by six bacteria species. Resistance towards the cell wall biosynthesis inhibiting antibiotic vancomycin was expressed by six bacteria species. Their resistance is in agreement with the literature where similar findings of ARB and antibiotic residues have been discovered in foods (Perreten et al., 1998; Kyriacou et al., 2008; Guo et al., 2017; Singla et al., 2018; Campedelli et al., 2019). Further, no isolated bacteria species expressed resistance towards tetracycline. This could be considered as a positive result since tetracycline is the second most sold antibiotic group for humans and the fourth most sold for animals in Sweden (Folkhälsomyndigheten, 2020). However, tetracycline resistant bacteria have been isolated from dairy products in earlier studies and resistance has been observed in bacteria from the same genus as those bacteria isolated in this study

(Hankin et al., 1979; Danielsen and Wind, 2003; Devirgiliis et al., 2013; Özdikmenli Tepeli and Demirel Zorba, 2018).

5.1. Unpasteurized milk

Unpasteurized milk is known to be able to carry harmful pathogenic bacteria (CDC, 2017) and in agreement with our study, ARB have earlier been discovered in unpasteurized milk (Hankin et al., 1979; Tóth et al., 2020). In our study, five ARB species were identified.

R. ornithinolytica is a gram- negative bacterium that belongs to the family *Enterobacteriaceae* (Hajjar et al., 2020). *R. ornithinolytica* cause mastitis among livestock and have been detected as an ARB in cow and buffalo milk, showing resistance towards antibiotics such as tetracycline and ampicillin (Abd Ali and Alali, 2017). This is in agreement with our study where *R. ornithinolytica* expressed resistance towards ampicillin. However, *R. ornithinolytica* have also been detected in fish (Tominaga, 2018) and vegetables (Luo et al., 2017). In this study, *R. ornithinolytica* was one of the isolated bacteria species that expressed resistance towards the majority of the different antibiotics. *R. ornithinolytica* expressed resistance towards two cell wall biosynthesis inhibiting antibiotics, ampicillin and vancomycin, and three protein biosynthesis inhibiting antibiotics erythromycin, streptomycin and clindamycin.

S. maltophilia is a gram- negative bacterium associated with human infections (El-Prince et al., 2019). The bacteria have been documented in milk and dairy products. *S. maltophilia* can be found in a variety of soil, plant and aquatic environments and has been connected to outbreaks of clinical mastitis (Ohnishi et al., 2012). Bovine mastitis *S. maltophilia* isolates have expressed resistance to many classes of antibiotics including β-lactams, kanamycin and gentamicin. S. *maltophilia* resistant towards chloramphenicol have also been detected in humans (Çıkman et al., 2016). The results from this study give that that *S. maltophilia* expressed resistance towards the cell wall biosynthesis inhibiting antibiotic vancomycin and two protein biosynthesis inhibiting antibiotics, i.e. erythromycin and clindamycin. The ZD for the resistant bacteria was 0 mm thus indicating full resistance towards vancomycin, erythromycin and clindamycin. For the other antibiotics the ZD ranged between 12-26 mm (see Table 4). No ZD data were available to determine if *S. maltophilia* was resistant to any other antibiotic. EUCAST (2021) stated that the resistance of *S. maltophilia* is a major problem to the antibiotic class of aminoglycosides. The results of this study did not indicate such resistance, but instead the isolates were fully resistant towards the antibiotic classes glycopeptides, macrolides and lincosamides.

P. fulva is a gram-negative bacterium that can cause human, animal and plant diseases (Uchino et al., 2001; Ruiz-Roldán et al., 2020). Earlier detections of

Pseudomonas spp. in unpasteurized milk were reported by Almeida et al. (2017). Antibiotic resistance of *Pseudomonas spp*. among healthy animals has been reported by Ruiz-Roldán et al (2020) and antibiotic resistant *Pseudomonas spp.* isolates from water were observed by Vaz-Moreira et al. (2012). Meng et al. (2020) reported discovery of antibiotic resistant *Pseudomonas spp.* from unpasteurized milk. Their study showed that *Pseudomonas spp.* isolates were resistant to one of the antibiotics used in this study, chloramphenicol. The results from this study indicated that *P. fulva* expressed intermediate susceptibility towards chloramphenicol. However, Quintieri et al. (2019) reported discoveries of *Pseudomonas spp.* isolated from different dairy foods and their resistance towards different classes of antibiotics. In their study, *P. fulva* isolated from bulk tank milk was resistant to β-lactams and aminoglycosides antibiotics. In our study, no specified ZD data were available for *P. fulva.* Instead, ZD data for *P. aeruginosa* were used. *P. aeruginosa* is a bacterium from the same genus and its antibiotic resistance is well characterized and widely reported (Vaz-Moreira et al., 2012). In this study, *P. fulva* was one of the isolated bacteria species that expressed resistance towards the greatest number of different antibiotics. *P. fulva* expressed resistance towards the cell wall biosynthesis inhibiting antibiotics, ampicillin and vancomycin and towards two protein biosynthesis inhibiting antibiotics, i.e. erythromycin and clindamycin. However, it needs to be taken into consideration that ZD were used for another bacterium from the same genus, and *P. aeruginosa* might express susceptibility differently compared to *P. fulva.*

P. pentosaceus is a gram-positive LAB that is widely occurring in food and dairy environments (Singla et al., 2018). The bacteria have also been used as probiotic and biological growth promotor in animal feed (Danielsen et al., 2007). *Pediococcus spp.* have a documented intrinsic resistance towards vancomycin, kanamycin, gentamicin and streptomycin (Singla et al., 2018). These results are in agreement with our study, where *P. pentosaceus* expressed resistance towards the cell wall inhibiting antibiotic vancomycin and towards the three protein biosynthesis inhibiting antibiotics gentamicin, kanamycin and streptomycin. In contrast to the results in our study, where it was susceptible, P. *pentosaceus* has in an earlier study expressed resistance towards tetracycline (Hummel et al., 2007).

L. paracasei is a gram-positive LAB that originate from the gastrointestinal tract of humans and animals (Ledina et al., 2018). It can enter the environment and contaminate raw materials. *L. paracasei* has previously been found in milk and is reported as a non-starter LAB of relevance in the production of certain cheese varieties (Budinich et al., 2011; Stefanovic et al., 2018). *L. paracasei* can also be found as a probiotic strain in commercial fermented milk products (Sánchez et al., 2017) where vancomycin and kanamycin resistant *L. paracasei* have been isolated from yoghurt (Kyriacou et al., 2008). Also, vancomycin, kanamycin and tetracycline resistant *L. paracasei* have been isolated from raw milk cheese and

other fermented milk products. (Ledina et al., 2018; Guo et al., 2017). These findings are in agreement with results from our study. *L. paracasei* isolated from unpasteurized milk expressed resistance towards the protein biosynthesis inhibiting antibiotic kanamycin and the cell wall inhibiting antibiotic vancomycin. *L. paracasei* also expressed intermediate susceptibility towards tetracycline. Even more, the results from this study indicate that *L. paracasei* is resistant towards streptomycin and gentamicin. Similar resistance has also been documented among other lactobacilli strains (Guo et al., 2017).

The ARB species *R. ornithinolytica* and *S. maltophilia* identified from unpasteurized milk relate to mastitis whilst *P. fulva* relate to other animal diseases. This may suggest that the livestock at the selected farm were treated with antibiotics against infectious diseases in the past, thus gaining related ARB. The bacteria may have gained their resistance through HGT. The resistant bacteria may already exist in the cow's udder but may also transmit into the milk during and after milking through contamination as milk is excellent medium for bacteria growth (Marjan et al., 2014).

5.2. Fresh cheese

One single ARB species was identified in the fresh cheese sample. *P. brenneri* is a gram-negative bacterium from the group *Pseudomonas flourescens* and the genus *Pseudomonas*. In an earlier study, *P. brenneri* was isolated from natural mineral waters (Baïda et al., 2001). Strains within the same *P. flourescens* group have been isolated from raw milk (Almeida et al. 2017) and were reported to be involved in depredation of milk in dairy products (Arslan et al., 2011). Antibiotic resistant strains of *P. flourescens* have been isolated from Turkish white cheese, raw milk, soft cheese and Italian mozzarella (Quintieri et al., 2019), with multiple antibiotic resistance among the isolates. Among the antibiotics, the *P. flourescens* strains expressed resistance towards ampicillin and chloramphenicol. This is in agreement with the results of the current study, where *P. brenneri* expressed resistance towards ampicillin and chloramphenicol. The results from this study also indicate that *P. brenneri* expressed resistance towards the two-protein biosynthesis inhibiting antibiotics erythromycin and clindamycin and towards the cell wall biosynthesis inhibiting antibiotic vancomycin. However, no ZD data were available for *P. brenneri* and instead ZD data for *P. aeruginosa* were used.

According to the manufacturer of the fresh cheese used for this study both the milk and the cream were pasteurized prior production (Arla, 2021). This may explain why few bacteria were discovered in the fresh cheese. Contamination during the production might have contributed to the presence of the bacteria isolated from the product. In this study, *P. brenneri* was one of the isolated bacteria species that expressed resistance towards a total of five different antibiotics. However, it

needs to be taken into consideration that ZD were used for a bacterium from the same genus, and *P. aeruginosa* might express susceptibility differently than *P. brenneri.*

5.3. Ripened hard cheese

A single ARB species was identified, *L. paracasei.* As earlier mentioned (see 5.1. Unpasteurized milk), *L. paracasei* has been reported as a non-starter LAB of relevance for cheese production (Budinich et al., 2011; Cogan, 2011). Antibiotic resistant *L. paracasei* have been isolated from different dairy products (Kyriacou et al., 2008; Ledina et al., 2018; Guo et al., 2017). The major sources of non-starter LAB in cheese is the raw milk and/or the dairy factory environment (Cogan, 2011). Lactobacilli can survive both pasteurization and the high cooking temperature (52ºC) used to make specific hard cheeses. It can also adapt to an environment with limited amount of nutrients, e.g. during the later stages of cheese ripening (Stefanovic et al., 2018). In this study, *L. paracasei* expressed resistance towards the cell wall biosynthesis inhibiting antibiotic vancomycin and three protein biosynthesis inhibiting antibiotics gentamicin, kanamycin and streptomycin. In Sweden, the consumption of cheese is 19 kg per person a year, where approximately 70% of the consumption consist of hard cheese (Naturvårdsverket 2020; Jordbruksverket[3] 2020). This may be of concern since *L. paracasei* is one of the most common non-starter LAB in manufacturing of cheese (Fitzsimons et al., 1999; Settanni and Moschetti, 2010; Gobbetti et al., 2015; Stefanovic et al., 2018).

5.4. Pasteurized milk

ARB have previously been isolated from pasteurized milk (Hankin et al., 1979; Marjan et al., 2014). Also in this study, three antibiotic resistant bacteria species were identified in pasteurized milk.

One identified bacteria species was *B. licheniformis*. The bacteria is gram-positive and from the genus *Bacillus* (Clements et al., 2002). Limited ZD data were available for *B. licheniformis*, however, the results from this study indicated that *B. licheniformis* did not express any resistance.

K. varians is a gram-positive bacterium from the genus *Kocuria* and the family *Micrococcus* (Savini et al., 2010). The bacteria has been isolated from unpasteurized cow milk and cheese and has displayed strong lipolytic and proteolytic activities (Centeno et al., 2017; Rodríguez-Alonso et al., 2011). The bacteria have also been isolated from salami, where it sometimes is used for flavor development, and from the milk of water deer's (O'Mahony et al., 2001; Demeyer and Toldrá, 2004; Li et al., 2017). In addition, bacteria from the genus *Kocuria* were

isolated from human skin (Grice et al., 2008). *K. varians* has ability to grow at low temperatures (8ºC) (Rodríguez-Alonso et al., 2011) which might cause problems during the cold storage of the milk. *K. varians* isolates have shown the ability to produce variacin, an antibiotic belonging to the same class of antimicrobial peptides as nisin, which can inhibit food-borne pathogens such as *Staphylococcus aureus, Bacillus cereus, Enterococcus and Clostridium botulinum* (O'Mahony et al., 2001; Chamba and Irlinger, 2004). No ZD data were available for *K. varians*, however, two of the isolates expressed full resistance towards the protein biosynthesis inhibiting antibiotic clindamycin. If a general ZD for the family of *Micrococcus* were used (Eady et al., 2000) to determine the susceptibility of the isolates towards tetracycline and gentamicin, the result would suggest that *K. varians* expressed susceptibility.

S. hominis is a gram-positive, coagulase negative bacterium from the genus *Staphylococcus* (Taponen and Pyörälä, 2009). Staphylococci are the bacteria most commonly isolated from milk of dairy cows suffering from subclinical mastitis (Taponen and Pyörälä, 2009; Condas et al., 2017; Wald et al., 2019). *S. hominis* with expressed β-lactam antibiotic resistance has been isolated from bovine milk from cows with intramammary infections (Condas et al., 2017; Kim et al., 2019). Also in this study, *S. hominis* expressed resistance towards a β-lactam antibiotic, ampicillin. *S. hominis* has also been isolated from human skin (Kloos and Schleifer, 1975). However, bacteria within this genus were also isolated from cheese made from unpasteurized milk and raw meat products, expressing resistance towards chloramphenicol, tetracycline, gentamicin, kanamycin and erythromycin (Perreten et al., 1998). This study indicates that *S. hominis* express resistance towards the protein biosynthesis inhibiting antibiotics gentamicin, kanamycin and clindamycin, which partially agrees with the findings of Perreten et al (1998).

Considering that pasteurization kills 99.99% of the bacteria (Tóth et al., 2020), and that both *K. varians* and *S. hominis* have been isolated from human skin, re-contamination of the milk after pasteurization and during further processing of the milk is one possible explanation for the presence of the bacteria in the milk. In Sweden, low pasteurization of the raw milk at 72ºC for 15 seconds is the most common heat treatment (Livsmedelsverket 2020). In water, DNA degradation starts at 90ºC (Tóth et al., 2020). The bacterial composition of milk is affected by the pasteurization, but the antibiotic resistant genes may still be present. These genes might further be incorporated into other bacterial genomes through HGT. Findings of ARB in pasteurized milk may therefore be of concern since the average milk consumption in Sweden is approximately 70 L per person a year (Naturvårdsverket 2020).

5.5. Fermented milk

Five bacteria species isolated from fermented milk expressed antibiotic resistance, *R. terrigena, P. fulva, S. hominis, R. planticola* and *L. delbrueckii.*

R. planticola and *R. ornithinolytica* (see 5.1 Unpasteurized milk for *R. ornithinolytica*) are two closely related species which are difficult to differentiate with MALDI-TOF MS (Hajjar et al., 2020; de Jong et al., 2013). *R. planticola* and *R. terrigena* are found in the soil and water environment and belong to the genus *Raoultella* from the family *Enterobacteriaceae* (Koc et al., 2013; Izard et al., 1981). These gram-negative bacteria are also found in the rumen, in bovine fecal and in the dairy environment (Massé et al., 2020). Antibiotic resistant *R. planticola* have been isolated from clinical mastitis cases in dairy cattle and expressed resistance towards streptomycin (Bagley et al., 1981; Massé et al., 2020). Multidrug resistant *R. planticola* have been isolated from river water (Koc et al., 2013).

In this study, both *R. planticola* and *R. terrigena* expressed resistance towards the protein biosynthesis inhibiting antibiotics gentamicin, kanamycin and streptomycin. Resistance towards streptomycin is in agreement with Massé et al (2020). According to the manufacturer, the fermented milk product yoghurt used for this study is pasteurized at high temperature (95ºC, 10 min) (Arla 2021; Livsmedelsverket 2020). The bacteria may therefore have been transmitted through contamination, e.g. after pasteurization during manufacture and handling of the milk in the dairy where personnel might follow inadequate hygienic practice. However, improper pasteurization and unacceptable levels of hygiene indicators may also increase the chance to introduce these bacteria into the milk (Marjan et al., 2014).

P. fulva was earlier discussed (see 5.1 Unpasteurized milk*). Pseudomonas spp*. are environmental bacteria and are recognized as major food spoilage microorganism (Arslan et al., 2011). These bacteria have been isolated from dairy products where they expressed resistance towards different classes of antibiotics (Arslan et al., 2011; Quintieri et al., 2019). *P. fulva* harboring a pool of resistance genes can transmit these to other bacteria in animals or humans (Quintieri et al., 2019). Results from this study show that *P. fulva* express resistance towards the protein biosynthesis inhibiting antibiotic gentamicin*,* thus may be able to transmit its resistance towards gentamicin to bacteria in humans when yoghurt is consumed. The expressed resistance and number of *P. fulva* isolates from fermented milk differ from the *P. fulva* isolates from unpasteurized milk. A single isolate of *P. fulva* was identified from fermented milk, expressing resistance towards a single antibiotic, gentamicin. Six isolates of *P. fulva* were identified in unpasteurized milk, expressing resistance towards four different antibiotics (see 5.1 Unpasteurized milk). The difference might be due to that the bacteria are killed by the high temperature heat treatment and low pH during the production of the yoghurt.

S. hominis have earlier been discussed (see 5.4 Pasteurized milk). The bacteria have been isolated from human skin and as β-lactam antibiotic resistant in bovine milk from cows suffering from mastitis (Kloos and Schleifer, 1975; Kim et al., 2019). However, *S. hominis* isolated from yoghurt in this study expressed resistance towards the two-protein biosynthesis inhibiting antibiotics gentamicin and kanamycin which partially differs from the *S. hominis* isolated from pasteurized milk (see 5.4 Pasteurized milk).

L. delbrueckii is a gram-positive LAB and one of the main starter LAB in cultures used in the production of yoghurt (Kyriacou et al., 2008; Ashraf and Shah, 2011; Wang et al., 2019). *L. delbrueckii* subs. *bulgaricus, L. delbrueckii* subs. *lactis and L. delbrueckii* subs. *delbrueckii* have all been isolated from yoghurt with expressed antibiotic resistance towards vancomycin, kanamycin, clindamycin, tetracycline, chloramphenicol, streptomycin, erythromycin and ampicillin (Kyriacou et al., 2008; Campedelli et al., 2019). This is in agreement with the results from this study where the isolated *L. delbrueckii* expressed resistance towards the protein biosynthesis inhibiting antibiotic gentamicin, kanamycin and streptomycin. *L. delbrueckii* used in yoghurt production can harbor antibiotic resistance genes from the resistant bacteria in unpasteurized milk and disseminate the resistance through yoghurt (Wang et al., 2019). Bacteria that are added as starter cultures in the production of food should thus not be antibiotic resistant from food safety and public health perspectives. When resistant *L. delbrueckii* are used as starters in yoghurt production, a large number of cells will enter the intestine and interact with the indigenous intestinal microbiota in the human and may therefore transfer the antibiotic resistant genes by HGT (Devirgiliis et al., 2013; Wang et al., 2019). This might be of concern since the consumption of fermented milk products in Sweden is approximately 27 L per person a year (Jordbruksverket[3] 2020). Screening for antibiotic resistant starter cultures in fermented milk products should therefore be routinely done in order to ensure food safety.

S. salivarius and *M. luteus* were two isolates from the yoghurt sample which expressed no resistance and for which no ZD were available to determine the susceptibility. *M. luteus* are naturally found in milk and can result in spoilage of milk products (Parkash et al., 2007). However, *M. luteus* isolates would express susceptibility towards gentamicin and tetracycline if general ZD (Eady et al., 2000) for the family of *Micrococcus* were used to determine the susceptibility. According to the results from this study, *M. luteus* might be resistant towards kanamycin and streptomycin. The ZD around these two antibiotic disks (11 mm respectively) corresponded to antibiotic resistant ZD that were expressed from the other bacteria species isolated from the yoghurt (see Table 8).

5.6. General discussion

The disk diffusion method used in this study for screening of antibiotic resistant bacteria makes it easy to study the bacterial susceptibility. However, this method limits the possibility to determine the minimum inhibitory concentration (MIC) of the antibiotic that will inhibit the visible growth of the bacteria. This would be of interest to determine in order to judge the performance of an antibiotic with a certain concentration towards the isolated ARB. Another limitation with this project was that only one batch of each product was screened for ARB. The variability between batches is in this case unknown. Therefore, in order to ensure the significance of our results, more batches produced during the year would need to be screened. However, the hypothesis that no ARB will be found in the dairy products, earlier stated in this paper, unfortunately must be rejected.

5.7. Further research

In order to validate the results beyond a single batch, further research could focus on extensive screening of ARB with additional antibiotics, additional batches from the same product and other dairy products from the same and/or different Swedish dairy brands. More research is needed to elucidate the scale of the problem and the effect of ARB and antibiotic residues obtained in Swedish dairy products. Further research could also consider a screening of ARB from unpasteurized milk that is qualified as safe for use in manufacturing production.

6. Conclusion

The purpose of this study was to make a screening of antibiotic resistant bacteria in unpasteurized milk and dairy products available on Swedish market. The study aimed to analyze bacteria from pasteurized milk, fresh cheese, ripened hard cheese, fermented milk and unpasteurized milk. Nine different antibiotics that belong to some of the most common used in human and veterinary medicine were tested using the isolated bacteria.

It was found that unpasteurized milk and all dairy products contained antibiotic resistant bacteria. In total, eleven different antibiotic resistant bacteria species were isolated and identified in unpasteurized milk and the dairy products. These bacteria species expressed resistance towards eight of the antibiotics investigated apart from tetracycline. Three of the bacteria species were resistant towards the majority of the tested antibiotics. In unpasteurized milk and fermented milk five bacteria species that expressed antibiotic resistance were isolated. Two antibiotic resistant bacteria species were isolated in pasteurized milk. In fresh cheese and ripened hard cheese one antibiotic resistant bacteria species was isolated. Antibiotic resistant LAB was isolated in unpasteurized milk, ripened hard cheese and fermented milk. Seven bacteria species expressed resistance towards the protein biosynthesis inhibiting antibiotic gentamicin. Kanamycin, streptomycin, clindamycin and vancomycin were other antibiotics to which resistance was commonly expressed among the bacteria species.

This project was designed as a pilot study. Single batches of unpasteurized milk and dairy products were included, which limits the possibilities to validate the results. Therefore, in order to ensure the significance of our results more batches produced during the year would need to be screened. Further research should also include other dairy products and antibiotics in order to elucidate how frequent the occurrence of antibiotic resistant bacteria in Swedish dairy products is. The results from this study indicate that a more thorough screening of antibiotic resistant bacteria might be necessary in order to ensure the food safety before Swedish dairy products reach the retail market.

References

Abd Ali, M., Alali, Z., (2017). Antibiotic Resistance of Raoultella Ornithinolytica Strains Isolatd from Some Cow's and Buffalo's Milk Samples Causing Subclinical Mastitis in Misan City. doi: 10.131.40/RG.2.2.34450.96960

Alexander, T.W., Inglis, G.D., Yanke, L.J., Topp, E., Read, R.R., Reuter, T., McAllister, T.A., (2010). Farm-to-fork characterization of Escherichia coli associated with feedlot cattle with a known history of antimicrobial use. International Journal of Food Microbiology 137, 40–48. https://doi.org/10.1016/j.ijfoodmicro.2009.11.008

Almeida, K., Bruzaroski, S., Zanol, D., Melo, M., Santos, J., Aragon-Alegro, L., Botaro, B., Santana, E., (2017). Pseudomonas spp. and P. fluorescens: Population in refrigerated raw milk. Ciência Rural 47. https://doi.org/10.1590/0103-8478cr20151540

Andersson, D.I., Hughes, D., (2010). Antibiotic resistance and its cost: is it possible to reverse resistance? Nature Reviews Microbiology 8, 260–271. https://doi.org/10.1038/nrmicro2319

Andersson, D.I., Hughes, D., (2011). Persistence of antibiotic resistance in bacterial populations. FEMS Microbiology Reviews 35, 901–911. https://doi.org/10.1111/j.1574-6976.2011.00289.x

Arla (2021) Recept, matinspiration och goda produkter [WWW Document], n.d. . Arla. URL https://www.arla.se/ (accessed 3.2.21).

Arslan, S., Eyi, A., Özdemir, F., (2011). Spoilage potentials and antimicrobial resistance of Pseudomonas spp. isolated from cheeses. Journal of Dairy Science 94, 5851–5856. https://doi.org/10.3168/jds.2011-4676

Aryal, S., (2020). Mueller Hinton Agar (MHA) | Culture Media, 2020. . Microbe Notes. URL https://microbenotes.com/mueller-hinton-agar-mha/ (accessed 1.13.21).

Ashraf, R., Shah, N.P., (2011). Selective and differential enumerations of Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium spp. in yoghurt — A review. International Journal of Food Microbiology 149, 194–208. https://doi.org/10.1016/j.ijfoodmicro.2011.07.008

Awad, H.M., EL, Shahed, K.Y.I., Aziz, R., Sarmidi, M.R., El, Enshasy, H.A., (2012). Antibiotics as Microbial Secondary Metabolites: Production and Application. Jurnal Teknologi 59. https://doi.org/10.11113/jt.v59.1593

Bagley, S.T., Seidler, R.J., Brenner, D.J., (1981). Klebsiella planticola sp. nov.: A new species of enterobacteriaceae found primarily in nonclinical

environments. Current Microbiology 6, 105–109. https://doi.org/10.1007/BF01569013
Baïda, N., Yazourh, A., Singer, E., Izard, D., (2001). Pseudomonas brenneri sp. nov., a new species isolated from natural mineral waters. Research in Microbiology 152, 493–502. https://doi.org/10.1016/S0923-2508(01)01223-2
Bernal-Rosas, Y., Osorio-Muñoz, K., Torres-García, O., (2015). Pseudomonas aeruginosa: an emerging nosocomial trouble in veterinary. Revista MVZ Córdoba 20, 4937–4946. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0122-02682015000400009
Biemer, J.J., (1973). Antimicrobial Susceptibility Testing by the Kirby-Bauer Disc Diffusion Method. Ann Clin Lab Sci 3, 135–140. http://www.annclinlabsci.org/content/3/2/135
Blair, J.M.A., Webber, M.A., Baylay, A.J., Ogbolu, D.O., Piddock, L.J.V., (2015). Molecular mechanisms of antibiotic resistance. Nature Reviews Microbiology 13, 42–51. https://doi.org/10.1038/nrmicro3380
Brady, M.s., Katz, S.e., (1988). Antibiotic/Antimicrobial Residues in Milk1. Journal of Food Protection 51, 8–11. https://doi.org/10.4315/0362-028X-51.1.8
Budinich, M.F., Perez-Díaz, I., Cai, H., Rankin, S.A., Broadbent, J.R., Steele, J.L., (2011). Growth of Lactobacillus paracasei ATCC 334 in a cheese model system: A biochemical approach. Journal of Dairy Science 94, 5263–5277. https://doi.org/10.3168/jds.2009-2512
Bush, K., Mobashery, S., (1998). How β-Lactamases Have Driven Pharmaceutical Drug Discovery, in: Rosen, B.P., Mobashery, S. (Eds.), Resolving the Antibiotic Paradox: Progress in Understanding Drug Resistance and Development of New Antibiotics, Advances in Experimental Medicine and Biology. Springer US, Boston, MA, pp. 71–98. https://doi.org/10.1007/978-1-4615-4897-3_5
Campedelli, I., Mathur, H., Salvetti, E., Clarke, S., Rea, M.C., Torriani, S., Ross, R.P., Hill, C., O'Toole, P.W., (2019). Genus-Wide Assessment of Antibiotic Resistance in Lactobacillus spp. Appl. Environ. Microbiol. 85. https://doi.org/10.1128/AEM.01738-18
Castanon, J.I.R., (2007). History of the Use of Antibiotic as Growth Promoters in European Poultry Feeds. Poultry Science 86, 2466–2471. https://doi.org/10.3382/ps.2007-00249
CDC, (2017). Raw Milk Questions and Answers [WWW Document]. Centers for Disease Control and Prevention. URL https://www.cdc.gov/foodsafety/rawmilk/raw-milk-questions-and-answers.html (accessed 2.24.21).
CDC, (2020) Measuring Outpatient Antibiotic Prescribing [WWW Document]. URL https://www.cdc.gov/antibiotic-use/community/programs-measurement/measuring-antibiotic-prescribing.html (accessed 1.20.21).

Centeno, J.A., Garabal, J.I., Docampo, F., Lorenzo, J.M., Carballo, J., (2017). Recovering traditional raw-milk Tetilla cheese flavour and sensory attributes by using Kocuria varians and Yarrowia lipolytica adjunct cultures. International Journal of Food Microbiology 251, 33–40. https://doi.org/10.1016/j.ijfoodmicro.2017.03.014

Chajęcka-Wierzchowska, W., Zadernowska, A., García-Solache, M., (2020). Ready-to-eat dairy products as a source of multidrug-resistant Enterococcus strains: Phenotypic and genotypic characteristics. Journal of Dairy Science 103, 4068–4077. https://doi.org/10.3168/jds.2019-17395

Chamba, J.-F., Irlinger, F., (2004). Secondary and Adjunct Cultures, in: Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (Eds.), Cheese: Chemistry, Physics and Microbiology, General Aspects. Academic Press, pp. 191–206. https://doi.org/10.1016/S1874-558X(04)80068-X

Charteris, W.P., Kelly, P.M., Morelli, L., Collins, J.K., (1998). Antibiotic Susceptibility of Potentially Probiotic Lactobacillus Species. Journal of Food Protection 61, 1636–1643. https://doi.org/10.4315/0362-028X-61.12.1636

Çıkman, A., Parlak, M., Bayram, Y., Güdücüoğlu, H., Berktaş, M., (2016). Antibiotics resistance of Stenotrophomonas maltophilia strains isolated from various clinical specimens. Afr Health Sci 16, 149–152. https://doi.org/10.4314/ahs.v16i1.20

Clements, L.D., Miller, B.S., Streips, U.N., (2002). Comparative Growth Analysis of the Facultative Anaerobes Bacillus subtilis, Bacillus licheniformis, and Escherichia coli. Systematic and Applied Microbiology 25, 284–286. https://doi.org/10.1078/0723-2020-00108

Croxatto, A., Prod'hom, G., Greub, G., (2012). Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiology Reviews 36, 380–407. https://doi.org/10.1111/j.1574-6976.2011.00298.x

Coates, A.R., Halls, G., Hu, Y., (2011). Novel classes of antibiotics or more of the same? Br J Pharmacol 163, 184–194. https://doi.org/10.1111/j.1476-5381.2011.01250.x

Cogan, T.M., (2011). Cheese | Microbiology of Cheese, in: Fuquay, J.W. (Ed.), Encyclopedia of Dairy Sciences (Second Edition). Academic Press, San Diego, pp. 625–631. https://doi.org/10.1016/B978-0-12-374407-4.00076-5

Compound Interest (2014). *A Brief Overview of Classes of Antibiotics 2014*. Compound Interest. URL https://www.compoundchem.com/2014/09/08/antibiotics/ (accessed 2.8.21).

Condas, L.A.Z., De Buck, J., Nobrega, D.B., Carson, D.A., Naushad, S., De Vliegher, S., Zadoks, R.N., Middleton, J.R., Dufour, S., Kastelic, J.P., Barkema, H.W., (2017). Prevalence of non-aureus staphylococci species causing intramammary infections in Canadian dairy herds. Journal of Dairy Science 100, 5592–5612. https://doi.org/10.3168/jds.2016-12478

Danielsen, M., Simpson, P.J., O'Connor, E.B., Ross, R.P., Stanton, C., (2007). Susceptibility of Pediococcus spp. to antimicrobial agents. Journal of Applied Microbiology 102, 384–389. https://doi.org/10.1111/j.1365-2672.2006.03097.x

Danielsen, M., Wind, A., 2003. Susceptibility of Lactobacillus spp. to antimicrobial agents. International Journal of Food Microbiology 82, 1–11. https://doi.org/10.1016/S0168-1605(02)00254-4

Demeyer, D., Toldrá, F., (2004). FERMENTATION, in: Jensen, W.K. (Ed.), Encyclopedia of Meat Sciences. Elsevier, Oxford, pp. 467–474. https://doi.org/10.1016/B0-12-464970-X/00160-4

de Jong, E., de Jong, A.S., Smidts-van den Berg, N., Rentenaar, R.J., (2013). Differentiation of Raoultella ornithinolytica/planticola and Klebsiella oxytoca clinical isolates by matrix-assisted laser desorption/ionization-time of flight mass spectrometry. Diagnostic Microbiology and Infectious Disease 75, 431–433. https://doi.org/10.1016/j.diagmicrobio.2012.12.009

Devirgiliis, C., Zinno, P., Perozzi, G., (2013). Update on antibiotic resistance in foodborne Lactobacillus and Lactococcus species. Front. Microbiol. 4. https://doi.org/10.3389/fmicb.2013.00301

Difco & BBL Manual, *Manual of microbiological culture media* 2nd edition (2003).https://legacy.bd.com/europe/regulatory/Assets/IFU/Difco_BBL/218561.pdf (accessed 12.23.20)

Duse, A., Waller, K.P., Emanuelson, U., Unnerstad, H.E., Persson, Y., Bengtsson, B., (2013). Farming practices in Sweden related to feeding milk and colostrum from cows treated with antimicrobials to dairy calves. Acta Vet Scand 55, 49. https://doi.org/10.1186/1751-0147-55-49

Eady, E.A., Coates, P., Ross, J.I., Ratyal, A.H., Cove, J.H., (2000). Antibiotic resistance patterns of aerobic coryneforms and furazolidone-resistant Gram-positive cocci from the skin surface of the human axilla and fourth toe cleft. Journal of Antimicrobial Chemotherapy 46, 205–213. https://doi.org/10.1093/jac/46.2.205

EFSA (2007), Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA - Opinion of the Scientific Committee, 2007. . EFSA Journal 5, 587. https://doi.org/10.2903/j.efsa.2007.587

El-Prince, E., Amin, W., Thabet, S., Hanna, M., (2019). Stenotrophomonas species in milk and some dairy products. https://www.researchgate.net/profile/Wallaa-Amin/publication/333855672_Stenotrophomonas_species_in_milk_and_some_dairy_products/links/5d091f6892851cfcc62297fd/Stenotrophomonas-species-in-milk-and-some-dairy-products.pdf

EUCAST (2020): Disk diffusion methodology vaersion 8.0. January 2020 [WWW Document], n.d. URL https://www.eucast.org/ast_of_bacteria/disk_diffusion_methodology/ (accessed 1.29.21).

EUCAST (2021): The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0, 2021. Clinical breakpoints and dosing of antibiotics [WWW Document], n.d. URL https://eucast.org/clinical_breakpoints/ (accessed 1.29.21).

Ferri, M., Ranucci, E., Romagnoli, P., Giaccone, V., (2017). Antimicrobial resistance: A global emerging threat to public health systems. Critical Reviews in Food Science and Nutrition 57, 2857–2876. https://doi.org/10.1080/10408398.2015.1077192

Fitzsimons, N.A., Cogan, T.M., Condon, S., Beresford, T., (1999). Phenotypic and Genotypic Characterization of Non-Starter Lactic Acid Bacteria in Mature Cheddar Cheese. Appl. Environ. Microbiol. 65, 3418–3426. https://doi.org/10.1128/AEM.65.8.3418-3426.1999

Folkhälsomyndigheten (2020), Swedres-Svarm 2019. Folkhälsomyndigheten [WWW Document], n.d. URL http://www.folkhalsomyndigheten.se/publicerat-material/publikationsarkiv/s/swedres-svarm-2019/ (accessed 3.3.21).

Garzón, V., Bustos, H., Pinacho, D., (2020). Personalized Medicine Review Personalized Medicine for Antibiotics: The Role of Nanobiosensors in Therapeutic Drug Monitoring. Journal of Personalized Medicine 10, 1–34. https://doi.org/10.3390/jpm10040147

Georgieva, R., Yocheva, L., Tserovska, L., Zhelezova, G., Stefanova, N., Atanasova, A., Danguleva, A., Ivanova, G., Karapetkov, N., Rumyan, N., Karaivanova, E., (2015). Antimicrobial activity and antibiotic susceptibility of Lactobacillus and Bifidobacterium spp. intended for use as starter and probiotic cultures. Biotechnol Biotechnol Equip 29, 84–91. https://doi.org/10.1080/13102818.2014.987450

Gobbetti, M., De Angelis, M., Di Cagno, R., Mancini, L., Fox, P.F., (2015). Pros and cons for using non-starter lactic acid bacteria (NSLAB) as secondary/adjunct starters for cheese ripening. Trends in Food Science & Technology 45, 167–178. https://doi.org/10.1016/j.tifs.2015.07.016

Grice, E.A., Kong, H.H., Renaud, G., Young, A.C., Bouffard, G.G., Blakesley, R.W., Wolfsberg, T.G., Turner, M.L., Segre, J.A., (2008). A diversity profile of the human skin microbiota. Genome Res. 18, 1043–1050. https://doi.org/10.1101/gr.075549.107

Griffiths, A.J., Miller, J.H., Suzuki, D.T., Lewontin, R.C., Gelbart, W.M., (2000). Transduction. An Introduction to Genetic Analysis. 7th edition. https://www.ncbi.nlm.nih.gov/books/NBK21760/

Guo, H., Pan, L., Li, L., Lu, J., Kwok, L., Menghe, B., Zhang, H., Zhang, W., (2017). Characterization of Antibiotic Resistance Genes from Lactobacillus Isolated from Traditional Dairy Products. Journal of Food Science 82, 724–730. https://doi.org/10.1111/1750-3841.13645

Gupta, A., Nelson, J.M., Barrett, T.J., Tauxe, R.V., Rossiter, S.P., Friedman, C.R., Joyce, K.W., Smith, K.E., Jones, T.F., Hawkins, M.A., Shiferaw, B.,

Beebe, J.L., Vugia, D.J., Rabatsky-Ehr, T., Benson, J.A., Root, T.P., Angulo, F.J., (2004). Antimicrobial Resistance among Campylobacter Strains, United States, 1997–2001. Emerg Infect Dis 10, 1102–1109. https://doi.org/10.3201/eid1006.030635

Gustavsson, E., (2003). SPR biosensor analysis of [beta]-lactam antibiotics in milk: devoplement and use of assays based on a [beta]-lactam receptor protein, Acta Universitatis Agriculturae Sueciae Agraria. Swedish Univ. of Agricultural Sciences, Uppsala.

Hajjar, R., Ambaraghassi, G., Sebajang, H., Schwenter, F., Su, S.-H., (2020). Raoultella ornithinolytica: Emergence and Resistance. Infect Drug Resist 13, 1091–1104. https://doi.org/10.2147/IDR.S191387

Hankin, L., Lacy, G.H., Stephens, G.R., Dillman, W.F., (1979). Antibiotic-resistant Bacteria in Raw Milk and Ability of Some to Transfer Antibiotic Resistance to Escherichia coli. Journal of Food Protection 42, 950–953. https://doi.org/10.4315/0362-028X-42.12.950

Holmes, R.K., Jobling, M.G., (1996). Genetics, in: Baron, S. (Ed.), Medical Microbiology. University of Texas Medical Branch at Galveston, Galveston (TX).

Hudzicki, J., (2009). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol [WWW Document]. URL https://www.asmscience.org/content/education/protocol/protocol.3189 (accessed 2.18.21).

Hummel, A.S., Hertel, C., Holzapfel, W.H., Franz, C.M.A.P., (2007). Antibiotic Resistances of Starter and Probiotic Strains of Lactic Acid Bacteria. Appl. Environ. Microbiol. 73, 730–739. https://doi.org/10.1128/AEM.02105-06

Imai, Y., Meyer, K.J., Iinishi, A., Favre-Godal, Q., Green, R., Manuse, S., Caboni, M., Mori, M., Niles, S., Ghiglieri, M., Honrao, C., Ma, X., Guo, J.J., Makriyannis, A., Linares-Otoya, L., Böhringer, N., Wuisan, Z.G., Kaur, H., Wu, R., Mateus, A., Typas, A., Savitski, M.M., Espinoza, J.L., O'Rourke, A., Nelson, K.E., Hiller, S., Noinaj, N., Schäberle, T.F., D'Onofrio, A., Lewis, K., (2019). A new antibiotic selectively kills Gram-negative pathogens. Nature 576, 459–464. https://doi.org/10.1038/s41586-019-1791-1

Izard, D., Ferragut, C., Gavini, F., Kersters, K., De ley, J., Leclerc, H., (1981). Klebsiella terrigena, a New Species from Soil and Water. International Journal of Systematic and Evolutionary Microbiology, 31, 116–127. https://doi.org/10.1099/00207713-31-2-116

Jett BD, Hatter KL, Huycke MM, Gilmore MS. Simplified agar plate method for quantifying viable bacteria. Biotechniques. (1997) Oct;23(4):648-50. doi: 10.2144/97234bm22. PMID: 9343684.

Jordbruksverket[1] (2020)- Marknaden för mjölk och mejeriprodukter - Jordbruksverket [WWW Document], n.d. URL http://djur.jordbruksverket.se/amnesomraden/handelmarknad/kottmjolkoc

hagg/marknadenforkottmjolkochagg/marknadenformjolkochmejeriprodukter.4.3a3862f81373bf24eab80001786.html (accessed 1.19.21).
Jordbruksverket[2] (2020) Swedish work against antibiotic resistance - a one health approach [WWW Document], n.d. URL https://webbutiken.jordbruksverket.se/sv/artiklar/ovr524.html (accessed 1.21.21).
Jordbruksverket[3] (2020), Direktkonsumtion efter Tabelluppgifter, Vara och År. [WWW Document], n.d. URL http://statistik.sjv.se/PXWeb/pxweb/sv/Jordbruksverkets%20statistikdatabas/Jordbruksverkets%20statistikdatabas__Konsumtion%20av%20livsmedel/JO1301K1.px/table/tableViewLayout1/ (accessed 2.28.21).
Jurinke, C., Oeth, P., van den Boom, D., (2004). MALDI-TOF mass spectrometry. Mol Biotechnol 26, 147163.https://doi.org/10.1385/MB:26:2:147
Kapoor, G., Saigal, S., Elongavan, A., (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. J Anaesthesiol Clin Pharmacol 33, 300–305. https://doi.org/10.4103/joacp.JOACP_349_15
Kim, S.-J., Moon, D.C., Park, S.-C., Kang, H.Y., Na, S.H., Lim, S.-K., (2019). Antimicrobial resistance and genetic characterization of coagulase-negative staphylococci from bovine mastitis milk samples in Korea. Journal of Dairy Science 102, 11439–11448. https://doi.org/10.3168/jds.2019-17028
Kim, Y.B., Seo, H.J., Seo, K.W., Jeon, H.Y., Kim, D.K., Kim, S.W., Lim, S.-K., Lee, Y.J., (2018). Characteristics of High-Level Ciprofloxacin-Resistant Enterococcus faecalis and Enterococcus faecium from Retail Chicken Meat in Korea. Journal of Food Protection 81, 1357–1363. https://doi.org/10.4315/0362-028X.JFP-18-046
Kloos, W.E., Schleifer, K.H., (1975). Isolation and Characterization of Staphylococci from Human Skin II. Descriptions of Four New Species: Staphylococcus warneri, Staphylococcus capitis, Staphylococcus hominis, and Staphylococcus simulans1. International Journal of Systematic and Evolutionary Microbiology, 25, 62–79. https://doi.org/10.1099/00207713-25-1-62
Koc, S., Kabatas, B., Icgen, B., (2013). Multidrug and Heavy Metal-Resistant Raoultella planticola Isolated from Surface Water. Bull Environ Contam Toxicol 91, 177–183. https://doi.org/10.1007/s00128-013-1031-6
Kohanski, M.A., Dwyer, D.J., Collins, J.J., (2010). How antibiotics kill bacteria: from targets to networks. Nature Reviews Microbiology 8, 423–435. https://doi.org/10.1038/nrmicro2333
Kyriacou, A., Tsimpidi, E., Kazantzi, E., Mitsou, E., Kirtzalidou, E., Oikonomou, Y., Gazis, G., Kotsou, M., (2008). Microbial content and antibiotic susceptibility of bacterial isolates from yoghurts. International Journal of Food Sciences and Nutrition 59, 512–525. https://doi.org/10.1080/09637480701565729

Ledina, T., Mohar-Lorbeg, P., Golob, M., Djordjevic, J., Bogovič-Matijašić, B., Bulajic, S., (2018). Tetracycline resistance in lactobacilli isolated from Serbian traditional raw milk cheeses. J Food Sci Technol 55, 1426–1434. https://doi.org/10.1007/s13197-018-3057-6

Li, Z., Wright, A.-D.G., Yang, Y., Si, H., Li, G., (2017). Unique Bacteria Community Composition and Co-occurrence in the Milk of Different Ruminants. Sci Rep 7. https://doi.org/10.1038/srep40950

Livsmedelsverket (2020) [WWW Document], n.d. URL Pastöriserad mjölk https://www.livsmedelsverket.se/livsmedel-och-innehall/mat-och-dryck/mjolk-och-mejeriprodukter/pastoriserad-mjolk (accessed 3.1.21).

Livsmedelsverket (2021) [WWW Document], n.d. URL https://www.livsmedelsverket.se/livsmedel-och-innehall/oonskade-amnen/lakemedelsrester (accessed 2.18.21).

LRF -Svensk mjölk i siffror - [WWW Document], (2020). Lantbrukarnas Riksförbund. URL https://www.lrf.se/om-lrf/organisation/branschavdelningar/lrf-mjolk/svensk-mjolk-i-siffror/ (accessed 1.19.21). LRF, 2020)

Luo, J., Yao, X., Lv, L., Doi, Y., Huang, X., Huang, S., Liu, J.-H., (2017). Emergence of mcr-1 in Raoultella ornithinolytica and Escherichia coli Isolates from Retail Vegetables in China. Antimicrobial Agents and Chemotherapy 61. https://doi.org/10.1128/AAC.01139-17

Marjan, S., Kanta Das, K., Kishore Munshi, S., Noor, R., (2014). Drug-resistant bacterial pathogens in milk and some milk products. Nutrition & Food Science 44, 241–248. https://doi.org/10.1108/NFS-05-2013-0061

Marshall, B.M., Levy, S.B., (2011). Food Animals and Antimicrobials: Impacts on Human Health. Clinical Microbiology Reviews 24, 718–733. https://doi.org/10.1128/CMR.00002-11

Martin, N.H., Trmčić, A., Hsieh, T.-H., Boor, K.J., Wiedmann, M., (2016). The Evolving Role of Coliforms As Indicators of Unhygienic Processing Conditions in Dairy Foods. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.01549

Massé, J., Dufour, S., Archambault, M., (2020). Characterization of Klebsiella isolates obtained from clinical mastitis cases in dairy cattle. Journal of Dairy Science 103, 3392–3400. https://doi.org/10.3168/jds.2019-17324

Meng, L., Liu, H., Lan, T., Dong, L., Hu, H., Zhao, S., Zhang, Y., Zheng, N., Wang, J., (2020). Antibiotic Resistance Patterns of Pseudomonas spp. Isolated From Raw Milk Revealed by Whole Genome Sequencing. Front. Microbiol. 11. https://doi.org/10.3389/fmicb.2020.01005

MicroDok microbiology, (2018). HISTORY, SOURCES & MECHANISM OF ACTION OF ANTIBIOTICS. Microbiology. URL https://microdok.com/antibiotics-resistance/ (accessed 2.4.21).

Munita, J.M., Arias, C.A., (2016). Mechanisms of Antibiotic Resistance. Microbiol Spectr 4. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015

Naturvårdsverket (2020), Statistik - Svensk konsumtion och produktion av mjölk och ost [WWW Document], n.d. Naturvårdsverket. URL https://www.naturvardsverket.se/Sa-mar-miljon/Statistik-A-O/Mjolk-och-ost-konsumtion-och-produktion-i-Sverige/ (accessed 2.28.21)

Ndahetuye, J.B., (2019) Mastitis in dairy cowns in Rwanda, prevalence, aetiology, antimicrobial resistance, molecular epidemiology and effects on milk quality. Doctoral thesis Swedish University of Agricultural Sciences, Uppsala 2019. https://pub.epsilon.slu.se/16465/7/ndahetuye_jb_191127.pdf

Normanno, G., Corrente, M., La Salandra, G., Dambrosio, A., Quaglia, N.C., Parisi, A., Greco, G., Bellacicco, A.L., Virgilio, S., Celano, G.V., (2007). Methicillin-resistant Staphylococcus aureus (MRSA) in foods of animal origin product in Italy. International Journal of Food Microbiology 117, 219–222. https://doi.org/10.1016/j.ijfoodmicro.2007.04.006

OECD.org - OECD (2016) Antimicrobial resistance- policy insights, URL https://www.oecd.org/ (accessed 1.19.21).

Ohnishi, M., Sawada, T., Marumo, K., Harada, K., Hirose, K., Shimizu, A., Hayashimoto, M., Sato, R., Uchida, N., Kato, H., (2012). Antimicrobial susceptibility and genetic relatedness of bovine Stenotrophomonas maltophilia isolates from a mastitis outbreak. Letters in Applied Microbiology 54, 572–576. https://doi.org/10.1111/j.1472-765X.2012.03246.x

O'Mahony, T., Rekhif, N., Cavadini, C., Fitzgerald, G.F., (2001). The application of a fermented food ingredient containing 'variacin', a novel antimicrobial produced by Kocuria varians, to control the growth of Bacillus cereus in chilled dairy products. Journal of Applied Microbiology 90, 106–114. https://doi.org/10.1046/j.1365-2672.2001.01222.x

Oxoid[1] - Product Detail MRS [WWW Document], n.d. URL http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0361&c=UK&lang=EN&org=&print=Y (accessed 12.22.20).

Oxoid[2] - Product Detail M17 [WWW Document], n.d. URL http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0785&cat=&c=UK&lang=EN (accessed 12.23.20).

Oxoid[3] - Product Detail MSPR [WWW Document], n.d. URL http://www.oxoid.com/uk/blue/prod_detail/prod_detail.asp?pr=cm0085&c=uk&lang=en&org=&print=N (accessed 12.23.20).

OXOID (2013): Disc diffusion supplemental tables, Performance standards for antimicrobial susceptibility testing. http://www.oxoid.com

Parkash, M., Rajasekar, K., Karmegam, N., (2007). Bacterial Population of Raw Milk and Their Proteolytic and Lipolytic Activities 4.

Perreten, V., Giampà, N., Schuler-Schmid, U., Teuber, M., (1998). Antibiotic Resistance Genes in Coagulase-negative Staphylococci Isolated from Food. Systematic and Applied Microbiology 21, 113–120. https://doi.org/10.1016/S0723-2020(98)80014-3

Piddock, L.J.V., (2006). Multidrug-resistance efflux pumps ? not just for resistance. Nature Reviews Microbiology 4, 629–636. https://doi.org/10.1038/nrmicro1464

Pyörälä, S., (2009). Treatment of mastitis during lactation. Irish Veterinary Journal 62, S40. https://doi.org/10.1186/2046-0481-62-S4-S40

Quintieri, L., Fanelli, F., Caputo, L., (2019). Antibiotic Resistant Pseudomonas Spp. Spoilers in Fresh Dairy Products: An Underestimated Risk and the Control Strategies. Foods 8. https://doi.org/10.3390/foods8090372

Rodríguez-Alonso .P, J.A. Centeno, J.I. Garabal., (2011) Biochemical study of industrially produced Arzúa-Ulloa semi-soft cows' milk cheese: effects of storage under vacuum and modified atmospheres with high-nitrogen contents Int. Dairy J., 21 (2011), pp. 261-271 http://www.chiriottieditori.it/images/stories/IJFS%20archivio/IJFS202.pdf#page=14

Ruiz-Roldán, L., Rojo-Bezares, B., de Toro, M., López, M., Toledano, P., Lozano, C., Chichón, G., Alvarez-Erviti, L., Torres, C., Sáenz, Y., (2020). Antimicrobial resistance and virulence of Pseudomonas spp. among healthy animals: concern about exolysin ExlA detection. Scientific Reports 10, 11667. https://doi.org/10.1038/s41598-020-68575-1

Sandrin, T.R., Goldstein, J.E., Schumaker, S., (2013). MALDI TOF MS profiling of bacteria at the strain level: A review. Mass Spectrometry Reviews 32, 188–217. https://doi.org/10.1002/mas.21359

Sánchez, E., Nieto, J.C., Vidal, S., Santiago, A., Martinez, X., Sancho, F.J., Sancho-Bru, P., Mirelis, B., Corominola, H., Juárez, C., Manichanh, C., Guarner, C., Soriano, G., (2017). Fermented milk containing Lactobacillus paracasei subsp. paracasei CNCM I-1518 reduces bacterial translocation in rats treated with carbon tetrachloride. Scientific Reports 7, 45712. https://doi.org/10.1038/srep45712

Sefton, A.M., (2002). Mechanisms of Antimicrobial Resistance. Drugs 62, 557–566. https://doi.org/10.2165/00003495-200262040-00001

Settanni, L., Moschetti, G., (2010). Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. Food Microbiology 27, 691–697. https://doi.org/10.1016/j.fm.2010.05.023

Shahbandeh. M (2020*)* Cow milk production worldwide, 2019 [WWW Document], n.d. . Statista. URL https://www.statista.com/statistics/263952/production-of-milk-worldwide/ (accessed 1.18.21).

Sharma, V.K., Johnson, N., Cizmas, L., McDonald, T.J., Kim, H., (2016). A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. Chemosphere 150, 702–714. https://doi.org/10.1016/j.chemosphere.2015.12.084

Sigma-Aldrich[1] (2020), MRS Agar 2020 69964 [WWW Document], n.d. . Sigma-Aldrich. URL https://www.sigmaaldrich.com/catalog/product/sial/69964 (accessed 1.13.21).

Sigma-Aldrich[2] (2021), Mueller-Hinton Agar 2021 2 97580 [WWW Document], n.d. . Sigma-Aldrich. URL https://www.sigmaaldrich.com/catalog/product/sial/97580 (accessed 1.13.21).

Singhal, N., Kumar, M., Kanaujia, P.K., Virdi, J.S., (2015). MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front Microbiol 6. https://doi.org/10.3389/fmicb.2015.00791

Singla, V., Mandal, S., Sharma, P., Anand, S., Tomar, S.K., (2018). Antibiotic susceptibility profile of Pediococcus spp. from diverse sources. 3 Biotech 8. https://doi.org/10.1007/s13205-018-1514-6

Stefanovic, E., Kilcawley, K.N., Roces, C., Rea, M.C., O'Sullivan, M., Sheehan, J.J., McAuliffe, O., (2018). Evaluation of the Potential of Lactobacillus paracasei Adjuncts for Flavor Compounds Development and Diversification in Short-Aged Cheddar Cheese. Front Microbiol 9. https://doi.org/10.3389/fmicb.2018.01506

Tamber, S., Hancock, R.E.W., (2003). On the mechanism of solute uptake in Pseudomonas. Front Biosci 8, s472-483. https://doi.org/10.2741/1075

Taponen, S., Pyörälä, S., (2009). Coagulase-negative staphylococci as cause of bovine mastitis—Not so different from Staphylococcus aureus? Veterinary Microbiology, Special Issue: Heifer and CNS Mastitis 134, 29–36. https://doi.org/10.1016/j.vetmic.2008.09.011

Thomas, C.M., Nielsen, K.M., (2005). Mechanisms of, and Barriers to, Horizontal Gene Transfer between Bacteria. Nature Reviews Microbiology 3, 711–721. https://doi.org/10.1038/nrmicro1234

Tominaga, T., (2018). Rapid detection of Klebsiella pneumoniae, Klebsiella oxytoca, Raoultella ornithinolytica and other related bacteria in food by lateral-flow test strip immunoassays. Journal of Microbiological Methods 147, 43–49. https://doi.org/10.1016/j.mimet.2018.02.015

Tóth, A.G., Csabai, I., Krikó, E., Tőzsér, D., Maróti, G., Patai, Á.V., Makrai, L., Szita, G., Solymosi, N., (2020). Antimicrobial resistance genes in raw milk for human consumption. Scientific Reports 10, 7464. https://doi.org/10.1038/s41598-020-63675-4

Uchino, M., Shida, O., Uchimura, T., Komagata, K., (2001). Recharacterization of *Pseudomonas fulva* Iizuka and Komagata 1963, and proposals of *Pseudomonas parafulva* sp. nov. and *Pseudomonas cremoricolorata* sp. nov. The Journal of General and Applied Microbiology 47, 247–261. https://doi.org/10.2323/jgam.47.247

van den Bogaard, A.E., Stobberingh, E.E., (2000). Epidemiology of resistance to antibiotics: Links between animals and humans. International Journal of Antimicrobial Agents 14, 327–335. https://doi.org/10.1016/S0924-8579(00)00145-X

Vaz-Moreira, I., Nunes, O.C., Manaia, C.M., (2012). Diversity and antibiotic resistance in Pseudomonas spp. from drinking water. Science of The Total

Environment 426, 366–374. https://doi.org/10.1016/j.scitotenv.2012.03.046
Växa Sverige, (2019). Behandlingsincidens med antibakteriella medel för systemiskt bruk i kokontrollanslutna besättningar, 2001-2019. https://www.vxa.se/globalassets/dokument/statistik/antibiotikaforbrukning-2001-2019.pdf
Wald, R., Hess, C., Urbantke, V., Wittek, T., Baumgartner, M., (2019). Characterization of Staphylococcus Species Isolated from Bovine Quarter Milk Samples. Animals (Basel) 9. https://doi.org/10.3390/ani9050200
Walsh, C., (2003). *Antibiotics: actions, origins, resistance*. Harvard Medical School, Boston, Massachusetts, USA. ASM Press, Washington DC
Wang, K., Zhang, H., Feng, J., Ma, L., Fuente-Núñez, C. de la, Wang, S., Lu, X., (2019). Antibiotic resistance of lactic acid bacteria isolated from dairy products in Tianjin, China. Journal of Agriculture and Food Research 1, 100006. https://doi.org/10.1016/j.jafr.2019.100006
Webber, M.A., Piddock, L.J.V., (2003). The importance of efflux pumps in bacterial antibiotic resistance. Journal of Antimicrobial Chemotherapy 51, 9–11. https://doi.org/10.1093/jac/dkg050
WHO (2014) | Antimicrobial resistance: global report on surveillance 2014 [WWW Document], n.d. . WHO. URL http://www.who.int/drugresistance/documents/surveillancereport/en/ (accessed 1.20.21).
WHO (2009) | Report of the 1st meeting of the WHO Advisory Group on integrated surveillance of antimicrobial resistance (AGISAR) Copenhagen 2009 [WWW Document], n.d . WHO. URL https://www.who.int/foodsafety/foodborne_disease/AGISAR_2009_report_final.pdf
Widyastuti, Y., Rohmatussolihat , Febrisiantosa, A., (2014). The Role of Lactic Acid Bacteria in Milk Fermentation. Food and Nutrition Sciences 5, 435–442. https://doi.org/10.4236/fns.2014.54051
Yale, M.W., Marquardt, J.C., (1940). Factors affecting the survival of Streptococcus pyogenes in cheese. Journal of Milk Technology 3, 326–333.
Özdikmenli Tepeli, S., Demirel Zorba, N.N., (2018). Antibiotic-resistant bacteria in milk and some dairy products. pp. 19–27.https://www.researchgate.net/publication/336471173_Antibiotic-resistant_bacteria_in_milk_and_some_dairy_products

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Appendix 1- Popular Scientific Summary

Do you consume any Swedish milk or dairy products? If you do, you are not alone. In Sweden we consume just over 70 L milk per person every year. We also consume around 19 kg of cheese and 27 L of fermented milk products per person every year. Milk and dairy products contain a lot of bacteria that are considered as safe and healthy for the consumers. Unfortunately, milk can easily be contaminated. Contamination of milk and dairy products might lead to consumption of bacteria that are not healthy for us. Some of the bacteria that can exist in milk are those who have developed a resistance against one of the worlds most used medicines, i.e. antibiotics.

If the cow has been treated with an antibiotic for some infection disease, the milk from that cow cannot be delivered to the dairy until it has been demonstrated that no antibiotic residues exist in the milk. Even if the milk is assumed to be free from antibiotic residues, there is still a risk that antibiotic resistant bacteria exist in the milk. The milk is heat-treated by pasteurization before it is used in dairy products. The unhealthy bacteria are killed by pasteurization. However, the pasteurization doesn't kill all other bacteria. This means that antibiotic resistant bacteria can survive pasteurization and exist in the milk which will later be used for dairy products. The antibiotic resistant bacteria in the milk can further spread their resistance to non-antibiotic resistant bacteria in the surrounding. The milk can also become contaminated with antibiotic resistant bacteria in the dairy. The contamination might occur after the pasteurization during manufacture processing and handling of the milk where personnel might follow inadequate hygienic practice.

Research have demonstrated that antibiotic resistant bacteria have been found in different foods around the world. Milk products are among the foods that have often been reported to contain antibiotic resistant bacteria. That is why this study searched for antibiotic resistant bacteria in Swedish milk and popular dairy products as hard cheese, fresh cheese, and yoghurt. Unpasteurized milk collected from a farm in Sweden was also analyzed in this study. Nine different antibiotics that belong to some of the most commonly used antimicrobial drugs in human and veterinary medicine were tested towards the bacteria.

The results from this study indicate that antibiotic resistant bacteria existed in unpasteurized milk and in Swedish dairy products. Elven bacteria species that expressed antibiotic resistance were found in the dairy products and the unpasteurized milk. All of these bacteria species were resistant to at least one antibiotic. Some bacteria species were resistant to the majority of the antibiotics investigated. This could be alarming as the consumer of dairy products might get antibiotic resistant bacteria in their body. The bacteria could further spread their resistance to other non-antibiotic resistant bacteria. Today, antibiotic resistance among pathogens that infect humans is of big concern. Overconsumption and misuse of antibiotics in both animals and humans have created a global problem with the spread of antibiotic resistance. The World Health Organization have stated that one of the biggest threats of the 21st century to both human and animal health is antibiotic resistance. Foods that are considered safe to consume should therefore not contain antibiotic resistant bacteria if we want to slow down the spread.

So, do you want to continue to consume dairy products now when you've read this? You should not worry. In this study, it was not possible to determine if antibiotic resistant bacteria always are present in dairy products. This since only one batch from each product was included in the study. Further research needs to be completed and include additional batches and other dairy products. First then we can determine whether antibiotic resistant bacteria from Swedish dairy products are a concern. However, it should be of interest to detect antibiotic resistant bacteria in the dairy products before they reach the Swedish retail market.

Appendix 2- Zone diameter raw data

Table 11. Raw ZD data for Raoultella ornithinolytica and Stenotrophomonas maltophilia isolated in unpasteurized milk on VRBD and MH agar

Taxonomy	Replicate	AMP	C	CN	E	K	S	TE	VA	DA	mm
Raoultella ornithinolytica											
	1	11	26	18	0	20	12	25	0	0	mm
	2	10	26	17	0	20	10	24	0	0	mm
Raoultella ornithinolytica											
	1	10	25	18	0	20	16	25	0	0	mm
	2	10	25	18	0	20	18	25	0	0	mm
Stenotrophomonas maltophilia											
	1	12	26	19	0	21	17	25	0	0	mm
	2	11	25	19	0	20	17	26	0	0	mm
Raoultella ornithinolytica											
	1	11	25	19	0	20	18	25	0	0	mm
	2	11	25	20	0	21	18	26	0	0	mm
Raoultella ornithinolytica											
	1	10	25	19	0	20	17	25	0	0	mm
	2	10	26	19	0	21	18	25	0	0	mm
Raoultella ornithinolytica											
	1	11	27	20	0	22	17	25	0	0	mm
	2	11	26	20	0	22	19	25	0	0	mm

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA)

Table 12. Raw ZD data for Pseudomonas fulva isolated in unpasteurized milk on M17 and MH agar

Taxonomy	Replicate	AMP	C	CN	E	K	S	TE	VA	DA	mm
Pseudomonas fulva											
	1	0	13	18	0	25	12	25	0	0	mm
	2	0	14	19	0	24	13	24	0	0	mm
Pseudomonas fulva											
	1	0	14	19	0	24	12	25	0	0	mm
	2	0	13	20	0	25	12	25	0	0	mm
Pseudomonas fulva											
	1	0	13	20	0	24	12	27	0	0	mm
	2	0	13	20	0	24	13	25	0	0	mm
Pseudomonas fulva											
	1	0	14	19	0	25	12	24	0	0	mm
	2	0	13	20	0	24	12	25	0	0	mm
Pseudomonas fulva											
	1	0	13	20	0	25	13	24	0	0	mm
	2	0	14	20	0	25	13	25	0	0	mm
Pseudomonas fulva											
	1	0	13	20	0	24	12	25	0	0	mm
	2	0	13	20	0	25	12	25	0	0	mm

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA)

Table 13. Raw ZD data for Pediococcus pentosaceus and Lactobacillus paracasei isolated in unpasteurized milk on MRS agar

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA)

Taxonomy	Replicate	AMP	C	CN	E	K	S	TE	VA	DA	mm
Pediococcus pentosaceus											
	1	33	34	0	34	0	0	40	0	26	mm
	2	33	35	0	36	0	0	40	0	26	mm
Pediococcus pentosaceus											
	1	20	28	0	25	0	0	20	0	26	mm
	2	20	27	0	25	0	0	17	0	25	mm
Pediococcus pentosaceus											
	1	27	33	0	32	0	0	33	0	29	mm
	2	23	32	0	35	0	0	25	0	26	mm
Lactobacillus paracasei											
	1	20	27	0	25	0	0	17	0	26	mm
	2	20	27	0	25	0	0	18	0	25	mm
Lactobacillus paracasei											
	1	23	30	0	25	0	0	17	0	27	mm
	2	23	30	0	27	0	0	16	0	25	mm
Pediococcus pentosaceus											
	1	20	25	0	25	0	0	20	0	26	mm
	2	20	27	0	25	0	0	20	0	25	mm

Table 14. Raw ZD data for Pseudomonas brenneri isolated in fresh cheese on M17 and MH agar

Taxonomy	Replicate	AMP	C	CN	E	K	S	TE	VA	DA	mm
Pseudomonas brenneri											
	1	0	10	20	0	22	15	32	0	0	mm
	2	0	9	20	0	22	15	34	0	0	mm
Pseudomonas brenneri											
	1	0	9	23	0	28	16	34	0	0	mm
	2	0	7	23	0	29	16	33	0	0	mm
Pseudomonas brenneri											
	1	0	7	24	0	27	16	33	0	0	mm
	2	0	7	23	0	29	18	31	0	0	mm
Pseudomonas brenneri											
	1	0	8	23	0	29	16	32	0	0	mm
	2	0	8	23	0	28	19	30	0	0	mm
Pseudomonas brenneri											
	1	0	10	21	0	24	15	37	0	0	mm
	2	0	7	20	0	23	15	36	0	0	mm
Pseudomonas brenneri											
	1	0	8	24	0	29	16	33	0	0	mm
	2	0	8	24	0	29	17	31	0	0	mm

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA)

Table 15. Raw ZD data for Lactobacillus paracasei isolated in ripened hard cheese on MRS agar

Taxonomy	Replicate	AMP	C	CN	E	K	S	TE	VA	DA	mm
Lactobacillus paracasei											
	1	30	33	0	35	0	0	35	0	30	mm
	2	35	40	0	35	0	0	36	0	26	mm
Lactobacillus paracasei											
	1	29	30	0	35	0	0	35	0	30	mm
	2	30	32	0	33	0	0	34	0	25	mm
Lactobacillus paracasei											
	1	26	30	0	31	0	0	35	0	26	mm
	2	30	30	0	32	0	0	35	0	25	mm
Lactobacillus paracasei											
	1	26	28	0	33	0	0	32	0	24	mm
	2	26	30	0	31	0	0	36	0	25	mm
Lactobacillus paracasei											
	1	31	34	0	35	0	0	35	0	25	mm
	2	30	32	0	32	0	0	35	0	25	mm
Lactobacillus paracasei											
	1	34	34	0	33	0	0	34	0	23	mm
	2	35	35	0	32	0	0	35	0	25	mm

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA)

Table 16. Raw ZD data for Kocuria varians, Staphylococcus hominis and Bacillus licheniformis isolated in pasteurized milk on M17 and MH agar

Taxonomy	Replicate	AMP	C	CN	E	K	S	TE	VA	DA	mm
Kocuria varians											
	1	62	39	24	49	17	20	45	25	40	mm
	2	60	38	22	50	20	23	44	23	40	mm
Kocuria varians											
	1	64	36	23	50	20	23	48	25	40	mm
	2	62	42	21	50	21	24	46	25	42	mm
Staphylococcus hominis											
	1	11	23	19	30	14	8	42	24	0	mm
	2	15	28	20	31	11	10	42	23	0	mm
Bacillus licheniformis											
	1	64	42	25	52	17	20	46	22	42	mm
	2	66	41	24	52	19	22	47	25	41	mm
Kocuria varians											
	1	12	30	20	30	15	8	41	21	0	mm
	2	17	32	20	30	15	7	41	22	0	mm
Kocuria varians											
	1	13	30	18	29	14	9	40	22	0	mm
	2	13	27	17	28	14	7	41	22	0	mm

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA)

Table 17. Raw ZD data for Streptococcus salavarius, Raoultella terrigena, Pseudomonas fulva, Micrococcus luteus, Staphylococcus hominis and Raoultella planticola isolated in fermented milk on M17 and MH agar

Taxonomy	Replicate	AMP	C	CN	E	K	S	TE	VA	DA	mm
Streptococcus salavarius											
	1	41	40	26	43	25	22	45	25	25	mm
	2	42	40	26	42	22	24	43	25	25	mm
Raoultella terrigena											
	1	50	40	14	46	9	12	47	27	50	mm
	2	50	40	15	47	8	11	47	26	50	mm
Pseudomonas fulva											
	1	40	35	14	38	10	11	43	24	25	mm
	2	40	32	12	38	10	12	37	23	25	mm
Micrococcus luteus											
	1	50	38	22	47	10	11	45	30	45	mm
	2	50	36	20	45	11	11	42	30	46	mm
Staphylococcus hominis											
	1	42	37	15	40	9	10	42	26	45	mm
	2	42	35	14	43	10	11	37	26	45	mm
Raoultella planticola											
	1	47	40	12	47	9	13	46	27	52	mm
	2	50	47	19	50	10	11	49	30	54	mm

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA)

Table 18. Raw ZD data for Lactobacillus delbrueckii isolated in fermented milk on MRS agar

Taxonomy	Replicate	AMP	C	CN	E	K	S	TE	VA	DA	mm
Lactobacillus delbrueckii											
	1	60	40	18	45	25	24	50	30	50	mm
	2	60	42	20	44	20	25	45	35	50	mm
Lactobacillus delbrueckii											
	1	55	44	12	45	11	11	45	35	44	mm
	2	52	45	12	43	10	9	42	34	43	mm
Lactobacillus delbrueckii											
	1	55	45	20	43	22	22	42	33	42	mm
	2	55	45	18	40	21	21	45	35	42	mm
Lactobacillus delbrueckii											
	1	50	42	12	40	0	10	40	33	40	mm
	2	50	41	12	42	0	10	41	33	42	mm
Lactobacillus delbrueckii											
	1	52	40	16	41	17	16	40	30	40	mm
	2	53	40	17	40	17	17	40	30	40	mm

Abbreviations: Ampicillin (AMP), Chloramphenicol (C), Gentamicin (CN), Erythromycin (E), Kanamycin (K), Streptomycin (S), Tetracycline (TE), Vancomycin (VA), Clindamycin (DA)